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PHYSIOLOGICAL REVIEWS

VOL. III JULY, 1923 No. 3

NEUTRALITY REGULATIONS IN THE BODY

D. WRIGHT WILSON

Laboratory of Physiological Chemistry, School of Medicine, University of Pennsylvania

VARIATIONS IN THE REACTION OF THE BLOOD. The normal processes taking place in the living cells can be carried out only if the hydrogen ion concentration of the fluids inside and outside of the cells is maintained constant within quite narrow limits. The blood in the body is almost always slightly alkaline (about pH 7.4). Although the limits of variation in reaction within which life is possible are still in doubt, blood as acid as pH 6.95 (Hasselbalch and Lundsgaard, 1912; Van Slyke, Austin and Cullen, 1922) and as alkaline as pH 9.00 (Dale and Evans, 1922) has been reported. In spite of the great amount of work which has been done in this field there are but few data available which may be compared indiscriminately owing to the technical difficulties involved in determining the hydrogen ion concentration (cH) of blood by the electrometric method in the presence of oxygen and carbon dioxide, and owing to the lack of accurate constants for use in calculations where indirect methods are employed.

The limits of variation in the normal individual which result from temporarily taxing the available mechanisms for maintaining neutrality are nearly as great as the extremes just mentioned. Barr, Himwich and Green (1923) reported a pH of 7.05 (recalculated)[1] in blood after short periods of strenuous muscular exercise. On the alkaline side, Davies, Haldane and Kennaway (1920) found a pH of about 7.85 (recalculated) after forced breathing.

The difference in pH between arterial and venous blood has been studied by several investigators. Parsons (1917) calculated the change in pH as 0.02. Peters, Barr and Rule's (1920–21) data show variations

[1] Data designated in this way have been recalculated using the "ordinate correction" of Doisy, Briggs, Eaton and Chambers (1922) and 6.15 as the value of pK_1 in Hasselbalch's equation.

of 0.01 to 0.04 (recalculated). Doisy, Briggs, Eaton and Chambers (1922) calculated variations from 0.013 to 0.037. Dale and Evans (1922) using their colorimetric method, found a difference of 0.10 between the arterial and venous blood of an anesthetized cat.

THE REACTION OF CELL FLUIDS. These figures are for blood only and they tell us nothing about the reaction of the tissue cells. The study of the reaction of the fluids within the tissues is difficult on account of the rapid changes which occur when the tissue is removed from its normal environment. It appears, however, that the reactions of the blood and tissue cells are not widely different (Koehler, Severinghaus and Bradley, 1922).

One might suppose, at first glance, that the study of cells, such as those of the stomach which secrete hydrochloric acid, would furnish some clue as to the degree of acidity which these cells are able to withstand. Gastric juice has a pH of 1.7 and is the most acid fluid found in the body. Comparing the work of Fitzgerald (1910), Harvey and Bensley (1912) and Collip (1921) one gains the impression that within the parietal cells hydrochloric acid is formed (by the interaction of chlorides, phosphates and carbonic acid), which unites with a weak base and the resulting salt (seemingly of colloidal nature) yields a solution which is only faintly acid. The compound passes into the lumen of the excretory duct and, as it passes toward the opening into the stomach, is hydrolyzed and the weak base reabsorbed. The final acidity is reached only as the liquid is about to flow out into the stomach, where the cells are protected by a mucous coating. The contents of the secreting cells are, however, never more than very faintly acid. Some such mechanism involving selective diffusion may come into play in the mammalian pancreas and liver, where strongly alkaline fluids are secreted and may even occur in connection with the formation of urine by the kidney. These secretions of specialized cells offer opportunities for studying certain physico-chemical reactions, such as selective permeability through membranes, but do not necessarily furnish any information concerning the variations of the reactions within the tissue cells themselves.

The most satisfactory comparison of cH inside and outside of cells has been made in studying plasma and corpuscles. Fluid within the corpuscles has been found to be more acid than the plasma by about pH 0.05 to 0.13 (for references see below). That the cH within the cells may be slightly different from the blood which bathes them is made possible by the fact that certain substances are not free to diffuse

through the cell membrane. Nevertheless, an equilibrium exists between the cH of the cell fluid and that of the blood so that an alteration of one will be reflected in the other. The maintenance of a normal hydrogen ion concentration of the blood is, therefore, necessary in order that the activities of the cells shall not be impeded by abnormal changes in their reaction. But, besides carrying out this important function, the normal reaction of the blood must be maintained in order that it may act as an efficient carrier of oxygen and carbon dioxide.

MECHANISMS FOR REMOVING BASE AND ACID. Since the normal reaction of the body is maintained within such narrow limits there must be effective mechanisms within the body for taking care of a temporary excess of either base or acid, and since acids are formed in many phases of metabolism the organism evidently must have considerable capacity for handling acids. In the oxidation of food materials to furnish the energy and heat of the body, proteins, fats and carbohydrates all furnish large quantities of carbonic acid, which, although a weak acid, would cause serious injury to the tissues if allowed to accumulate in too high a concentration. Besides yielding carbonic acid upon oxidation proteins yield appreciable quantities of phosphoric and sulphuric acid. After strenuous muscular activity lactic acid accumulates. Under abnormal conditions beta-oxybutyric acid and aceto-acetic acid may be formed in large quantities. Thus it may be seen that the animal organism is called upon to dispose of large amounts of acid under both normal and abnormal conditions and to dispose of them sufficiently rapidly so that the hydrogen ion concentration of the blood and tissues does not rise above $1 \times 10^{-6.9}$. The disposal of basic radicles (mainly sodium, potassium, calcium and magnesium) is necessitated by the fact that salts containing basic radicles are a necessary part of the normal diet and the accumulation of an excess of base in the body would result in increased alkalinity of the tissue fluids as well as severe disturbances in the osmotic pressure equilibrium.

The great importance of regulating the reaction of the body within narrow limits is indicated by the number of elaborate mechanisms available for maintaining a normal acid-base equilibrium. Acids and bases which tend to accumulate in the body are excreted. The volatile CO_2 is eliminated by way of the lungs while the non-volatile acids, such as sulphuric, phosphoric and hydrochloric, and the bases are eliminated by way of the kidneys in the form of salts. Some salts probably pass from the blood into the intestine and are eliminated in the feces.

The tissues and blood must be protected against any great change in reaction during the period between formation and elimination of acid. The buffers in the tissues and blood constitute the great source of protection in this connection. The proteins and phosphates are probably the main buffers concerned.

It should be emphasized that the $NaHCO_3$ of the blood is the agent which neutralizes non-volatile acids such as sulphuric and phosphoric. Upon entering the blood, they react with $NaHCO_3$ as they would in a test tube, forming the corresponding salt of the non-volatile acid and liberating carbon dioxide. In a test tube the gas quickly passes from the solution into the air and, in a similar way in the body, the extra carbon dioxide is eliminated by the lungs and thus the end result is that the $NaHCO_3$ in the blood has neutralized a strong acid with no appreciable change in the cH of the solution. The neutralization of carbonic acid itself is brought about, not by the $NaHCO_3$ of the blood, but by protein and phosphates which, acting as storehouses for base, give it up thus permitting the formation of bicarbonate with the carbonic acid as it enters from the tissues.

In tracing the method of disposal of acid or base which has accumulated in some tissue, the following mechanisms have been shown to be concerned. The buffers of the tissue protect while the material diffuses into the blood and adjacent tissue. The material is carried by the blood with little change in reaction owing to its buffers. Other tissues of the body absorb some of the excess from the blood, so that the quantity of acid or alkali which the body can retain without exceeding the normal limits of capacity of the buffers depends not only upon the quantity which may be handled by the blood but also by the tissue fluids of the whole body. (Palmer and Van Slyke, 1917; Barr and Himwich, 1923, a.) This safeguards the body against an accumulation of material in excess of its ability to immediately excrete it. Besides this removal of excess acid and alkali by the body fluids, the tissues are able to destroy certain bases and acids or sometimes to form compounds to neutralize them. Ammonia, which is produced by the deaminization of amino acids, is quickly converted into the neutral compound, urea, while many organic acids are oxidized to carbonic acid. Ammonia is formed by the kidney (Nash and Benedict, 1922) to combine with excess acidic radicles, and lactic acid may be formed to combine with an excess of base (Macleod and Knapp, 1918–19). Besides these chemical mechanisms which aid in handling excess acids or bases until they are excreted, a physical mechanism of fundamental importance is the blood flow, the

rate and volume of which determine the amount of material which can be removed from a localized area and transported to the excretory organs.

There are several possible paths for excretion of acids and bases. The quantities excreted in the perspiration are too small to warrant consideration. The excretion by way of the feces seems to have little relation to the acid-base equilibrium in the body. We may say then that the volatile carbonic acid formed in the body is excreted by way of the lungs, and the kidneys constitute the only other regulated mechanism for the elimination of acids and bases. The relative quantities of acids removed by these two paths are so seldom emphasized that it is of interest to note that the equivalent of 20 to 40 liters of normal acid are excreted by way of the lungs in 24 hours, while during the same time 50 to 150 cc. of normal acid are excreted by way of the kidneys. Not only is by far the larger part of the acid formed in the body eliminated by way of the lungs but it is eliminated without the loss of any base from the body.

THE ELIMINATION OF CARBONIC ACID. Stated in its simplest form, the elimination of carbonic acid depends on the diffusion of its anhydride, carbon dioxide, from the blood into the alveoli and lower air spaces of the lungs which are being washed out by an intermittent inspiration of atmospheric air and expiration of this air mixed with alveolar air which has diffused into it. Efficient aeration of the blood is made possible by a capillary network over the alveoli which permits a film of blood to come into close contact with the air in the alveoli, separated only by a very thin layer of tissue (said to be 0.004 mm. thick). The normal contact between air and blood seems to be sufficiently intimate to permit rapid diffusion of carbon dioxide and practically complete equalization of the partial pressure of carbon dioxide in the two media (Krogh and Krogh, 1909–10). There is about 0.04 per cent CO_2 in atmospheric air so that the partial pressure of CO_2 is about 0.3 mm. Hg. The normal partial pressure of CO_2 in the alveolar air and arterial blood is about 40 mm. Hg. It would appear that under normal conditions the aeration of the blood is dependent mainly upon the degree of aeration of the alveolar air spaces rather than upon the rate of diffusion of carbon dioxide from the blood into the alveoli.[2] It is quite probable that all of the alveolar air spaces of the lung are not being uniformly ventilated so that the partial pressure of CO_2 is higher in some regions than in others.

[2] Under certain pathological conditions these relations may not exist.

The blood flowing through various parts of the lung will, therefore, be brought into equilibrium with the different CO_2 tensions. For this reason it is best to say that the respiration is regulated so as to maintain a certain average tension of CO_2 in the alveoli which will produce in the mixed arterial blood flowing from the lung, a cH just sufficient to furnish the normal stimulus to the respiratory center (Haldane, 1922).

It is important to emphasize that even with a constant cH in arterial blood the partial pressure of CO_2 in blood and alveolar air may vary. This may be illustrated by the following equation:

$$CO_2 \text{ pressure} \rightleftarrows (CO_2 \text{ dissolved}) \rightleftarrows (H_2CO_3) \rightleftarrows (HCO_3) + (H)$$

which indicates that, with $H\overline{CO_3}$ present in blood, there must also be present some molecules of H_2CO_3 and molecules of dissolved CO_2 gas and a tendency for this gas to escape from the solution. This tendency can be expressed as partial pressure of CO_2 and will be demonstrated by the passage of CO_2 from the blood into air with which it is brought in contact, unless the partial pressure of CO_2 in the air is as great as the partial pressure of CO_2 in the blood.

If for any reason the concentration of $H\overline{CO_3}$ ions in the blood is decreased (as in diabetic acidosis) and the cH remains constant, there must be a corresponding decrease in concentration of each member of the above series, and a decrease in partial pressure of CO_2 in the blood. This will result in a decreased tension and a decreased percentage of CO_2 in the alveolar air. With the normal aeration of the alveolar air spaces the amount of CO_2 removed will be correspondingly smaller than normal. Therefore, with decreased $NaHCO_3$ in the blood, an increased ventilation will be necessary to cause the removal of a constant quantity of CO_2 formed by the oxidations in the body and at the same time to maintain a normal cH. With a high bicarbonate concentration in the blood, the alveolar CO_2 tension will be high if the cH remains normal and a diminished ventilation of the lungs will suffice to remove the necessary quantities of CO_2.

Increase in the frequency of respiration will increase the aeration of the active air spaces, i.e., the air spaces which are being well ventilated. An increase in the depth of respiration will increase the number or volume of air spaces being well ventilated. Each of these mechanisms together with an increase in blood flow through the lungs will permit the removal of increasing quantities of CO_2 from the body. Alterations of these three variables, therefore, offer almost unlimited opportunity for delicate adjustments whereby the loss of carbonic acid from the body can be controlled within very narrow limits.

CHANGES OCCURRING IN THE BLOOD. Before discussing the regulatory mechanisms which control the loss of carbonic acid, the changes which take place in the blood may be examined. Venous blood loses CO_2 in the lungs and tends to become more alkaline, and arterial blood takes up CO_2 in the tissues and tends to become more acid. A number of other reactions occur simultaneously which cause the changes in reaction to be diminished greatly and there results a system of transport which carries large quantities of carbonic and other acids, loading in the tissues and unloading in the lungs and kidneys with little change in cH in either place.

The study of these changes in blood has occupied the attention of many investigators for over fifty years. Recently the work has received a great impetus due to the development of new methods for determining the cH (Hasselbalch, 1911; Sörensen, 1912; Michaelis, 1914; Clark, 1922) and the oxygen and carbon dioxide concentration in blood (Barcroft, 1914; Van Slyke and Stadie, 1921; Haldane, 1922). The methods have been improved until the data are now becoming sufficiently exact to give an accurate insight into the mechanisms involved. Van Slyke and his associates (1917–23) and L. J. Henderson, (1920–21) have assembled data and proposed new and more exact mathematical methods of studying the complex reactions involved. Warburg (1922) has made a detailed application of new theories of solutions and activities of ions together with a consideration of the complications introduced by the heterogeneity of all biological fluids containing colloids and colloidal membranes, which will at least modify some of the quantitative aspects of the discussions.

Investigators are encouraged, however, to find that so much of the data may be explained within the desired limits of accuracy by a consideration of the simpler theoretical applications. The principal factors involved in the acid-base equilibrium of the blood have been summarized in an article by Van Slyke (1921a) in a previous volume of the PHYSIOLOGICAL REVIEWS. Since that article was published, many experiments have been carried out which furnish more exact data and extend the knowledge of these mechanisms.

THE HEMOGLOBIN-OXYHEMOGLOBIN CHANGE. Blood in passing through the lungs takes up oxygen until the partial pressure of oxygen dissolved in the fluid is the same (or nearly the same) as that in the alveolar air and the hemoglobin has been changed almost entirely into oxyhemoglobin. The oxygen tension in the tissues is less than that of fully oxygenated blood owing to the utilization of oxygen by the tissues

for oxidations which are going on to a greater or less extent at all times. For this reason as arterial blood passes through the tissue capillaries, oxygen leaves the blood and diffuses into the tissue cells. At the same time carbon dioxide diffuses from the tissues into the blood owing to the difference in partial pressure of the gas in the two systems. The diffusion of each gas goes on entirely independently of the other depending only on the respective diffusion constants and the variation in partial pressure of each gas in the two liquid systems. There are secondary reactions, however, which take place and have an important bearing on the neutrality regulation of the blood.

The diffusion of carbon dioxide into the blood would obviously increase the carbonic acid and cause an increase in cH of the fluid. However, as oxygen diffuses out of the blood and the oxygen tension is thereby reduced, oxyhemoglobin dissociates into oxygen and hemoglobin. The two chemical compounds, oxyhemoglobin and hemoglobin, have slightly different chemical properties. In solutions having the reaction of the blood both act like weak acids, but oxyhemoglobin is a stronger acid than hemoglobin. (K for oxyhemoglobin $= 1 \times 10^{-6.3}$; K for reduced hemoglobin $= 1 \times 10^{-6.9}$.) The loss of oxygen from the blood causes some of the acid oxyhemoglobin to change into the weaker acid, hemoglobin. This is entirely analogous to a solution of hydrochloric acid suddenly being transformed to acetic acid. The resulting solution in both cases would be more alkaline. But at the time when the blood tends to become more alkaline due to the change of oxyhemoglobin to hemoglobin, carbonic acid enters from the tissues.

As all of the above reactions are equilibrium reactions, the obvious corollary is that the entrance of carbon dioxide with a resulting increase in cH will tend to increase the dissociation of oxyhemoglobin and increase of oxygen tension, thereby facilitating the diffusion of oxygen from the blood into the tissues. Exactly the reverse of this mechanism is encountered in the lungs. The entrance of oxygen into the blood with consequent increase in oxyhemoglobin tends to increase the acidity of the solution, setting free some carbon dioxide from the bicarbonates, and to accelerate the diffusion of carbon dioxide into the alveolar air.

The series of reactions taking place among the various buffer mixtures in the blood has been adequately explained in Van Slyke's paper (1921, a) on the carbon dioxide carriers. From his discussion it will be evident that the major portion of the carbon dioxide carried from the tissues to the lungs is carried in the form of bicarbonate salts of the bases present in the blood. The base is furnished by the various buffer

mixtures of the blood, mainly by the hemoglobin. The oxyhemoglobin and reduced hemoglobin exist in the blood partly as free acids and partly as salts. As oxyhemoglobin is the stronger acid the proportion of salt to free acid is greater than in the case of reduced hemoglobin, at the same cH.

$$\frac{BHbO_2}{HHbO_2} > \frac{BHb}{HHb}$$

If oxyhemoglobin changes to reduced hemoglobin, some of the base may go to form bicarbonate without change in cH of the blood. Thus, in the tissues, as oxygen leaves the blood and CO_2 enters, a certain quantity of CO_2 will be neutralized without change in reaction of the blood (isohydrically). But if more CO_2 enters than can be neutralized by this reaction, the cH of the blood increases with a consequent "liberation" of base from all the buffers of the blood. As a result, in all of the ratios:

$$\frac{BHb}{HHb} \quad \frac{BHbO_2}{HHbO_2} \quad \frac{BBHPO_4}{BHHPO_4} \quad \frac{B\ protein}{H\ protein}$$

the numerators will be decreased and the denominators increased.

Van Slyke (1921a) calculated the extent to which the various buffer systems play a part in carrying carbon dioxide, using data from the papers of Christiansen, Douglas and Haldane (1914) and Joffe and Poulton (1920–21) and supplying the necessary approximations at places where the data were incomplete. His calculations indicated that the total absorption of carbon dioxide due to base furnished by hemoglobin accounts for 84 per cent to 94 per cent of the total carbon dioxide absorbed with a change in pH of 0.025 to 0.09.

Recently Doisy, Briggs, Eaton and Chambers (1922) have carried out more complete experiments to obtain an evaluation of the buffers of the blood. From determinations of the CO_2 absorption curves of oxygenated and reduced blood and a hemoglobin solution, and determinations of inorganic phosphate, they have calculated the carbon dioxide carrying power of the various systems. Their results, recorded in the table below are valuable in yielding a closer experimental proof of the value of the various buffers and are the most exact available at present. But, owing to the numerous assumptions necessary on account of lack of available data these calculations are only close approximations.

It may now be stated without a doubt that by far the greater part of the CO_2 entering the blood from the tissues is carried to the lungs as the

salt of base released from hemoglobin. Half or more of the exchange takes place due to the isohydric change of oxyhemoglobin to hemoglobin, while a considerable portion of base is furnished by these two compounds acting as buffers, in a solution becoming slightly more acid due to the entrance of CO_2.

THE SHIFT OF CHLORIDES BETWEEN CORPUSCLES AND PLASMA. It is of interest to note that hemoglobin, the most important buffer in the blood, is isolated from the tissues by being placed in the corpuscles from

TABLE 1

Carbon dioxide carried by buffer systems studied (Doisy, Briggs, Eaton and Chambers 1922)

	E. A. D.		W. H. C.		J. M.	
	Vol. per cent	Per cent of total	Vol. per cent	Per cent of total	Vol. per cent	Per cent of total
Total CO_2 carried for R. Q. of 0.75	2.32		4.23		5.08	
$BHCO_3$ carried isohydrically $BHbO_2 \rightleftarrows HHb$	1.233	53.1	2.262	53.5	2.72	53.5
$BHCO_3$ carried by change of pH						
By hemoglobin: $BHbO_2 \rightleftarrows HHbO_2$						
$BHb \rightleftarrows HHb$	0.439	18.9	1.070	25.3	1.384	27.2
By B_2HPO_4 in cells	0.010	0.43	0.012	0.3	0.013	0.25
By separated serum	0.089	3.84	0.198	4.7	0.142	2.8
CO_2 physically dissolved	0.249	10.7	0.511	12.1	0.657	12.9
Sum, per cent of total	2.020	87.0	4.053	96.0	4.196	97.0
Per cent of total CO_2 carried by hemoglobin	72.0		78.8		80.7	
	pH		pH		pH	
Arterial blood (pH values recalculated)	7.296		7.310		7.281	
Venous blood (pH values recalculated)	7.283		7.280		7.244	
Difference	0.013		0.030		0.037	

which it is unable to dialyze. This is peculiarly important for the higher organisms as they now exist because when in solution in the plasma it is quickly eliminated or destroyed. It disturbs the acid-base balance of a solution on account of its acidic properties. Blood after hemolysis is more acid than before (Milroy, 1917; Fridericia, 1920; Campbell and Poulton, 1920–21; Conway and Stephen, 1922), and while its potential CO_2 carrying power is not altered, it can carry less CO_2 at a particular cH. Conway and Stephen (1922) find that within

the physiological limits of CO_2 tension the pH inside the corpuscle is 0.13 lower than in the plasma (i.e., the corpuscles are 35 per cent more acid than the plasma). If hemoglobin were present dissolved in plasma there would undoubtedly be little possibility of varying the concentration owing to disturbances in the osmotic relations which would doubtless occur. As it is, the concentration of hemoglobin may be increased and decreased without any osmotic disturbances merely through an increase or decrease of the number of red cells per unit volume of blood.

Hemoglobin is inclosed within a cell membrane which is freely permeable to water and CO_2, but the red cells do not carry all of the CO_2 which the above discussion indicates is carried by base furnished by hemoglobin. A peculiar change is found to take place which has only recently been adequately explained and the physiological import is far from being completely appreciated. Schmidt (1867) and Zuntz (1867) independently discovered that serum from blood equilibrated with a high tension of CO_2 contained more titratable alkali than serum from the same blood equilibrated at a low tension of CO_2. Zuntz erroneously concluded that sodium was split off from the cell proteins and diffused out from the corpuscles into the serum under the influence of increasing CO_2 tensions and formed $NaHCO_3$ in the serum. H. Nasse (1878) found that the chlorine in serum was decreased when blood was treated with high tensions of CO_2. The work was confirmed by Gürber (1895), Hamburger (1902) and others, who also demonstrated that there was no transfer of cations and that other anions also diffused to some extent. There were, however, some observations indicating a transfer of cations which have been refuted by the recent work of Doisy and Eaton (1921) and of Mukai (1921). Doisy and Eaton determined that the source of confusion had been due to failure to consider the loss of water from the plasma occurring when blood is brought into equilibrium with an increased CO_2 tension. Taking this into account, they found by actual analysis that there was no transfer of sodium or potassium between plasma and corpuscles. Mukai found that the total amount of ash in serum remains constant if allowance is made for loss or gain of water, indicating that there is no exchange of cations.

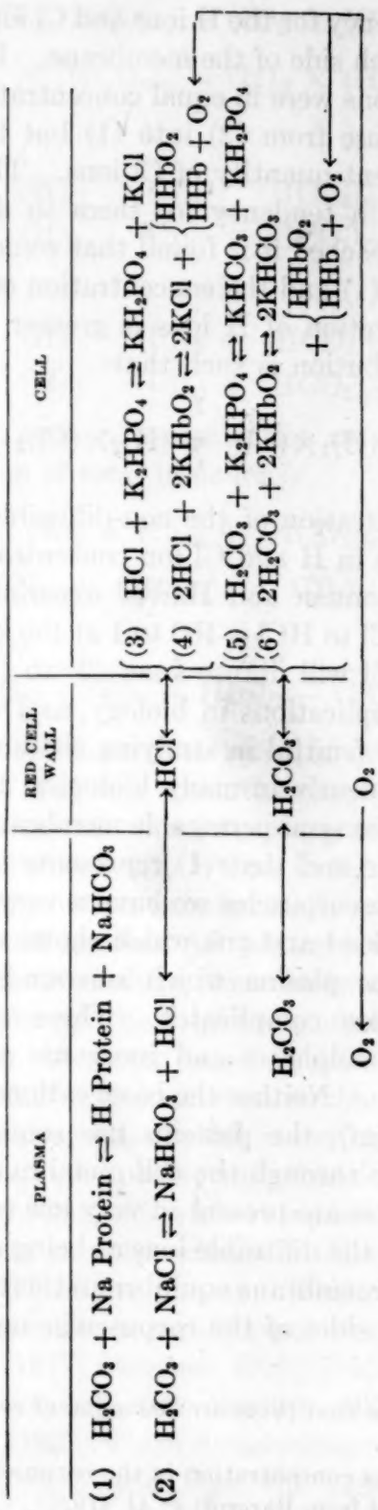
The concentration of hemoglobin in the corpuscle is about 30 per cent, while the concentration of proteins in the serum is about 8 per cent. When alkali is furnished by protein owing to an increased CO_2 tension (increased cH) much more alkali is furnished by the protein solution

in corpuscles. This tends to cause a much more rapid increase of $NaHCO_3$ and less rapid increase of cH inside the cell than in the plasma.

But what actually occurs is that there diffuses into the corpuscles not only H_2CO_3 (or CO_2) to react with the cell proteins and other buffers, but also HCl formed from the NaCl of the plasma. The loss of HCl permits the plasma bicarbonate to increase more rapidly than would be the case if only the plasma buffers were reacting. The loss of Cl from the plasma accounts for about three-fourths of the gain of plasma bicarbonate (Van Slyke and Cullen, 1917; Fridericia, 1920; L. J. Henderson, 1921; Warburg, 1922). Doisy, Eaton and Chouke (1922) calculate that, of the total base furnished to form plasma bicarbonate when blood absorbs CO_2 in changing reaction from pH 7.45 to 7.25, 16 per cent is furnished by the non-migrating serum buffers (plasma proteins, amino acids and organic acids). Of the remaining 84 per cent, 80 per cent is furnished by the migration of Cl into the corpuscles and the other 4 per cent probably by the migration of other acid radicles such as SO_4 (deBoer, 1917) and PO_4 (Doisy and Eaton, 1921). Doisy and Beckmann (1922) demonstrated that the Cl shift occurs in vivo by finding a change between arterial and venous blood. The transfer of Cl is such that about half of the added CO_2 is carried in the plasma and half in the cells. The actual reactions which take place in blood when CO_2 and O_2 are absorbed or given off is shown in the following chart taken from an article by Austin, Cullen, Hastings, McLean, Peters and Van Slyke (1922).

An explanation for the cause of the transfer of chloride between corpuscles and plasma has been found in applying a consideration of Donnan's theory of membrane equilibrium to the simpler phases of the problem. (Warburg, 1922; Barcroft, Bock, A. V. Hill, Parsons, Parsons, and Shoji, 1922; Van Slyke, Wu and McLean, 1923.) Donnan proposed in 1911 an explanation for the unequal distribution of diffusible ions on the two sides of a membrane which is impermeable to one ion in solution. Take for example, two solutions, one containing Na ions and Cl ions and the other H ions and Cl ions, separated by a membrane impermeable to the Na ions but through which H and Cl ions can pass freely. This may be represented by the following scheme.

(1)	(2)
Na	H
Cl	Cl


PLASMA
RED CELL WALL
CELL
(1) $H_2CO_3 + Na\ Protein \rightleftarrows H\ Protein + NaHCO_3$
(2) $H_2CO_3 + NaCl \rightleftarrows NaHCO_3 + HCl$
HCl
(3) $HCl + K_2HPO_4 \rightleftarrows KH_2PO_4 + KCl$
(4) $2HCl + 2KHbO_2 \rightleftarrows 2KCl + \begin{cases} HHbO_2 \\ HHb + O_2 \end{cases}$
(5) $H_2CO_3 + K_2HPO_4 \rightleftarrows KHCO_3 + KH_2PO_4$
(6) $2H_2CO_3 + 2KHbO_2 \rightleftarrows 2KHCO_3 + \begin{cases} HHbO_2 \\ HHb + O_2 \end{cases}$
H_2CO_3
O_2
H_2CO_3
O_2

There will be a tendency for the H ions and Cl ions to distribute themselves uniformly on each side of the membrane. Let us assume that at the beginning the Cl ions were in equal concentration in both solutions. Some H ions will diffuse from (2) into (1) but they can only diffuse along with an equivalent quantity of Cl ions. This leads to an excess of Cl ions in (1) and a tendency for them to diffuse back into (2). When equilibrium is reached it is found that some H and Cl ions have diffused from (2) into (1) and the concentration of Cl ions is greater in (1) while the concentration of H ions is greater in (2). Donnan has proved that the distribution is such that

$$(H)_1 \times (Cl)_1 = (H)_2 \times (Cl)_2$$

The larger the concentration of the non-diffusible ion Na, the greater will be the inequalities in H and Cl ion concentration on the two sides of the membrane. Donnan and Harris' experiments (1911) indicate that if the ratio of NaCl to HCl is 100 to 1 at the beginning, only about 1 per cent of the HCl will diffuse from (2) to (1). This theory undoubtedly has wide applications in biology, as Donnan has suggested, and should prove very fruitful in studying the unequal distribution of ions which occurs frequently in many biological tissues.

If we assume that the semi-permeable membrane is the membrane of the red blood corpuscle and that (1) represents the blood plasma and (2) the fluid within the corpuscles we have a very simplified picture of the equilibria in the blood and one which shows why the red cells can be more acid than the plasma which surrounds them. The actual conditions are far more complicated. There are similar equilibria between bicarbonates, sulphates and inorganic phosphates inside and outside of the corpuscle. Neither the basic cations (sodium, potassium, magnesium and calcium), the proteins nor some organic phosphorus compounds[3] can diffuse through the cell membrane. As the sulphates and diffusible phosphates are present in very low concentration we may, for simplicity, consider the diffusible ions as being only H, Cl and HCO_3. From Donnan's law of membrane equilibrium there will be a distribution of the ions on the two sides of the corpuscular membrane so that:[4]

[3] Bloor (1918) has shown that there are 200 mgm. of combined phosphoric acid per 100 cc. of corpuscles.

[4] The subletter indicates concentration in the corpuscles C or in the serum S. This discussion is modified from Barcroft et al. (1922).

$$(H)_C \times (Cl)_C = (H)_S \times (Cl)_S$$

$$(H)_C \times (HCO_3)_C = (H)_S \times (HCO_3)_S$$

Therefore:

$$\frac{(H)_C}{(H)_S} = \frac{(Cl)_S}{(Cl)_C} = \frac{(HCO_3)_S}{(HCO_3)_C}$$

and

$$\frac{(H)_C}{(H)_S} = \frac{(Cl)_S + (HCO_3)_S}{(Cl)_C + (HCO_3)_C}$$

As the (H) and serum protein concentration are very small (letting (B) represent concentration of total basic ions)

$$(B)_S = (Cl)_S + (HCO_3)_S$$

$$(B)_C = (Cl)_C + (HCO_3)_C + (Hb)_C + (PO_4)_C$$

$$\frac{(H)_C}{(H)_S} = \frac{(B)_S}{(B)_C - (Hb)_C - (PO_4)_C}$$

Van Slyke, Wu and McLean have recently shown that these as well as other relations hold true only when the concentrations are calculated in terms of gram equivalents of solute per grams of water as used in osmotic pressure calculations rather than in terms of gram equivalents of solute per volume of solution as customarily employed. As the water concentration in plasma is about 90 per cent and that of corpuscles 70 per cent the differences resulting from the two methods of calculation are considerable. Although the total concentration of base in the corpuscles (calculated on the water basis) is greater than in serum, the concentration of base actively concerned in the Donnan equilibrium is less owing to the large proportion which is combined with (balanced by) hemoglobin (and organic phosphate) and, therefore, has no influence on the equilibrium. According to the above equation, if the concentration of effective base is less in corpuscles, the cH of the corpuscles must be greater than the cH of the plasma. This conclusion based on Donnan's theory of membrane equilibrium is substantiated by many experiments (Milroy, 1917; Parsons, 1917; Fridericia, 1920; Joffe and Poulton, 1920–21; Campbell and Poulton, 1920–21; Evans, 1921b; Conway and Stephen, 1922) which demonstrate that the reaction in the corpuscle is actually more acid than the plasma. Warburg (1922) reports, that at pH 7.4, the pH of horse corpuscles is 0.09 to 0.13 less

than plasma and Conway and Stephen (1922) have shown with human blood that laked corpuscles have a pH 0.13 lower than plasma.

There is a tendency for each ion to distribute itself equally between solutions inside and outside of the corpuscle which is counteracted by the attractive forces of the non-diffusible ions, with a resultant unequal distribution when equilibrium is finally reached. When blood is brought in contact with a higher tension of CO_2, the proteins of the corpuscles furnish more base than do the proteins of the plasma so that the increase in (HCO_3) will be greater in corpuscles than in plasma. As (HCO_3) increases more rapidly in corpuscles the (H) within the corpuscles will rise less rapidly than in the plasma.

But with the unequal increase in (HCO_3) and (H) there is a disturbance of the ion equilibria and a shifting of the ions results. Some HCO_3 ions diffuse out of the corpuscles and about an equal number of Cl ions diffuse in. Other diffusible ions in the plasma (PO_4 and SO_4) take part in the interchange so that the rise of (HCO_3) in the plasma can be almost completely accounted for by loss of other ions from the plasma. Diffusion takes place until the increase of HCO_3 is distributed practically equally between corpuscles and plasma. The cause of the shifting of all the diffusible ions when only the equilibrium of (HCO_3) has been disturbed is, as stated by Warburg, in accordance with Donnan's theory and due to the tendency for the new equilibria to be established by a shifting which causes the same relative change in concentration (activity) of all diffusible ions.

These mechanisms are of importance in a consideration of the physiology of neutrality regulation. The corpuscles act as a storehouse for base which is furnished when oxygen is lost or carbon dioxide absorbed by the blood. By this mechanism the corpuscles neutralize most of the carbonic acid which enters the blood and, as a result, the acidity of the plasma increases much less rapidly than it otherwise would. Separated serum (serum with no corpuscles present) changes in reaction with changes of CO_2 tension much more rapidly than true serum (serum in contact with corpuscles) (Joffe and Poulton, 1920–21; Evans, 1921b).

The storage or release of H ions from the corpuscles with increase or decrease in CO_2 tension provides a much more delicate adjustment of reaction in the plasma and large changes in CO_2 tension can cause only small changes in its cH. With a limited change in cH of plasma the variation in CO_2 tension possible will depend on the amount of H ions which can be taken up. This will depend mainly on the relative quantity of corpuscles available and, of course, on the amount of the

buffers in each corpuscle. The latter variation, however, seems to be slight. The increased amount of buffer available as the percentage of corpuscles increases produces a steep CO_2 absorption curve: relatively smaller amounts of CO_2 absorbed at low CO_2 tensions and relatively larger amounts absorbed at high tensions. In the body, therefore, the CO_2 interchange can occur with smaller changes in reaction of the blood, or conversely, a greater CO_2 interchange is possible within the normal limits of blood reaction. The other extreme is evident when the quantity of corpuscles is low in a unit volume of blood. The CO_2 absorption curve approaches that of separated serum, so that the CO_2 interchange in the body causes greater changes in cH of the blood.

EXCHANGE OF WATER BETWEEN CORPUSCLES AND PLASMA. H. Nasse (1878) observed in experiments on the CO_2-carrying power of blood, that the corpuscles swelled when treated with increasing tensions of CO_2. This phenomenon was studied repeatedly but no adequate explanation was available until Spiro and Henderson (1909) proposed the explanation embodied in the following discussion.

With the change in reaction due to the increase or decrease in carbonic acid in the blood there are changes in the volume of the red cells brought about by alteration in the osmotic pressures of the fluids inside and out. When carbonic acid is added to blood the cH is increased and base is liberated from the protein salt to form ionized bicarbonate and unionized protein. The addition of a molecule of carbonic acid, therefore, causes the formation of three osmotically active particles in place of two. This reaction takes place to a greater extent in the corpuscles than in the plasma, as described above. The osmotic pressure of the corpuscles will, therefore, increase more rapidly than that of the plasma. As the corpuscles are elastic and permeable to water, water will enter and cause the corpuscles to swell until the osmotic pressures inside and out are the same. This reaction will take place regardless of the Cl shift which apparently has nothing to do with the total osmotic changes. Van Slyke, Wu and McLean (1923) have found that the water interchange can be explained quantitatively only when the concentrations of osmotically active particles are calculated in terms of gram equivalents per grams of water.

The change in volume of the cells causes an increase or decrease in water content of the surrounding plasma. The loss or gain of water by the plasma is not accompanied by loss or gain of salts (Doisy and Eaton, 1921; Mukai, 1921; Warburg, 1922) so their concentration changes with change in volume of the cells. These alterations in concentration have

been neglected until recently and have changed substantially some conclusions based on analyses of plasma.

The increase or decrease in volume is not great. Doisy and Eaton found in beef blood outside the body that the corpuscles occupied 43 volumes per cent in an atmosphere of 3 per cent CO_2 and 46 volumes per cent in pure CO_2. These authors studied the change in cell volume between arterial and venous blood and found wide variations with an average increase in venous blood of 0.35 volume per cent. It should be emphasized, however, that the changes taking place within the body are much more complicated than in a test tube and the regulation of the fluid interchange between blood and tissues cannot be explained so simply.

THE EQUILIBRIA IN THE BLOOD. In the normal interchange of gases in lungs and tissues all of the oxygen is not given up in the tissues nor all the carbon dioxide in the lungs. The large excess remaining in the blood at all times increases the flexibility of the regulating mechanisms and furnishes a great factor of safety for periods of unusual activity on the part of the organism.

The following table shows the distribution of the constituents which we have discussed above, in normal human blood under resting conditions. The concentrations are calculated on the volume basis.

ARTERIAL BLOOD	VENOUS BLOOD
(1) pH 7.30–7.40	7.27–7.37
(2) Free CO_2 1.5–4.0 vol. per cent (0.67–1.8 mM)	1.7–4.2 vol. per cent (0.76–1.87 mM)
CO_2 tension 22–63 mm. Hg.	25–65 mm. Hg.
(3) Combined CO_2 31–56 vol. per cent (14–25 mM)	36–61 vol. per cent (16–27 mM)
(4) Plasma Cl 370–390 mgm. per 100 cc. (105–110 mM)	360–370 mgm. per 100 cc. (102–107 mM)
(5) Percentage saturation of Hb 93–98	62–85
(6) O_2 tension 84–100 mm. Hg.	30–60 mm. Hg.
O_2 content 17–22 vol. per cent (7.6–9.8 mM)	11–16 vol. per cent (4.9–7.1 mM)

Under normal resting conditions these values show little tendency to change. The extreme constancy is brought about by the delicate regulation of the aeration of the blood in the lungs. It is important to note that, as L. J. Henderson (1921) has emphasized, a change in any one of the six different variables listed above causes a change in all or all but one of the others. The work of Haldane (1922) has demonstrated

that the variations in oxygen and carbon dioxide are the factors of fundamental importance to the organism and that, of these, the regulating mechanisms are mainly concerned with the carbon dioxide elimination.

THE STIMULUS OF THE RESPIRATORY CENTER. The ventilation of the lungs is regulated by the respiratory center in the medulla. Rhythmic expansion and contraction of the lungs is brought about by nerve impulses from the center to the muscles concerned with respiratory movements. The movements are adjusted in rate and depth so that the blood becomes sufficiently aerated. As the activity of the center and the resultant breathing are regulated by the blood which passes through it, it is important to determine what property of blood calls the center into action.

The specific substance which exercises control over the chemical regulation of respiration has been the subject of great discussion and frequent experimentation. Haldane and Priestley (1905) demonstrated conclusively that variations in the alveolar CO_2 tension called forth proportional responses of the respiratory center and consequent ventilation of the lungs and concluded that in this way the CO_2 tension of the respiratory center controlled the arterial CO_2 tension. The experiments of Winterstein (1911, 1915) and Hasselbalch (1912) on animals seemed to prove that the hydrogen ion concentration of the blood is the effective stimulus rather than the CO_2 tension. Laqueur and Verzár (1911), however, believed that the carbonic acid itself is a specific stimulus to the respiratory center rather than the H ions which the acid might produce. Since that time physiologists have been divided as to the actual agent which produces the stimulation, though the general view has been that the H ions are the effective stimulus.

Haggard and Y. Henderson (1919) have suggested another factor, "respiratory X" as an additional stimulus. Winterstein (1921) recently modified his theory proposing two ways in which the cH of the center may be influenced: first, by an increase or decrease in cH of the blood, and second, by the formation of acids in the center itself. The second postulate explains the response of the center in conditions of oxygen want as due to the formation of increased quantities of acid metabolic products in the center resulting in an increased cH.*

The problem of the specific stimulus has not been an easy one to solve. If the stimulation has something to do with the CO_2 tension of the solution, we must consider the possible action of CO_2, H_2CO_3, H ions and

* See also Gesell (1923).

HCO_3 ions (we cannot, at present distinguish between dissolved CO_2 and H_2CO_3 so they may be considered together). Changes in CO_2 tension are, therefore, usually accompanied by similar changes in cH. It is possible, however, to increase the CO_2 tension and at the same time decrease the cH but only by introducing other complicating factors such as Na ions and HCO_3 ions into the solutions. These other ions may stimulate the center or may change its irritability to other stimuli.

The following brief summary of evidence from recent experimentation may illustrate the difficulties attending this work. Hooker, Wilson and Connet (1917) showed that when the medulla of a dog was perfused with blood having a cH maintained by CO_2, the response of the respiratory center was much greater than when the perfusion was carried out with blood in which the cH was made the same by adding HCl. In these experiments, though the cH and Na ion concentration were kept constant, the blood which caused the greater stimulation contained higher concentrations of H_2CO_3 ($+CO_2$) and HCO_3. Scott's (1918–19) experiments, demonstrated that the respiratory center reacted similarly to increasing CO_2 tensions whether the reaction of the blood was normal or more alkaline than normal. In these experiments it would appear that the respiratory center responded to changes in H_2CO_3 ($+CO_2$) or HCO_3 regardless of the reaction of the blood. When breathing atmospheric air the ventilation was practically the same in each experiment, though the HCO_3 was higher in one than in the other. Collip (1920–21) showed that slow injections of $NaHCO_3$ caused increased breathing. Here it may be assumed that increased breathing was accompanied by an increase in Na, HCO_3 and H_2CO_3 and a decrease in cH of the blood. Dale and Evans (1922) injected $NaHCO_3$ until the reaction of the blood was pH 8.0 without producing apnea. As in the experiments of Collip, the low cH was accompanied by increased Na, HCO_3 and H_2CO_3. In both of these investigations the H_2CO_3 seemed to be less effective in the presence of high HCO_3 and low cH.

The experiments outlined above all appear to point to the conclusion that an increase in H_2CO_3 or HCO_3 stimulates the respiratory center and that it is relatively insensitive to variations in cH as such. It should be noted that all of these studies were made on anesthetized or decerebrate animals, and on the alkaline side of the normal reaction of the blood.

Seemingly contradictory evidence has been obtained by studies on men with apparently normal respiratory centers. In ordinary types of "acidosis" the blood may contain much less than the normal concen-

tration of H_2CO_3 and HCO_3 with the cH and respiration both probably slightly increased. On the other hand, after bicarbonate feeding, the blood is high in H_2CO_3 and HCO_3 while the respiration is depressed and the cH probably lowered somewhat. The experiments of Haggard and Y. Henderson (1919) are in agreement with the above. After forced breathing, apnea results with low H_2CO_3, low HCO_3 and low cH.

From the available data it seems apparent that both cH and the concentration of H_2CO_3 (or HCO_3) may play a part in the stimulus of the respiratory center. In attempting to correlate the seemingly conflicting evidence, the lack of available data on the permeability of the membranes between the blood plasma and the respiratory center renders an intelligent discussion difficult. Jacobs (1920, a, b) has shown that CO_2 or H_2CO_3 has great power of penetration through animal fluids and membranes. He has suggested that the stimulation of the respiratory center is due not only to the hydrogen ions from the blood acting at the cell surface but also to the action of the rapidly penetrating CO_2 in changing the cH within the center itself.

One may postulate that the equilibrium existing between the plasma and respiratory center may be somewhat analogous to the equilibrium existing between corpuscles and plasma, though we do not know at present what differences in permeability may exist in the two systems. If we assume an unequal distribution of bicarbonate and H ions between plasma and center and a mechanism similar to that found in blood, i.e., with increased alkalinity the difference in cH between center and plasma is increased, the following relations would occur. As bicarbonate is increased in the plasma the difference between center and plasma is increased. As H_2CO_3 increases to restore the cH and distributes itself proportionately between center and plasma the cH in the center would increase more rapidly due to the relatively lower HCO_3. The increased cH of the center would stimulate respiration increasing the elimination of CO_2 until the cH of the center was restored to normal, at which time the plasma would be more alkaline than normal. With low bicarbonate in the plasma the difference between plasma and center will be less and a normal cH of the center will be maintained when the cH of the plasma is greater than normal. With these assumptions all of the experiments are in agreement though the relations are, of course, far from being explained quantitatively.

The threshold of stimulation of the respiratory center by materials from the blood is definitely altered under certain conditions. When the center receives an insufficient amount of oxygen, respiration is greater

with the same concentration of CO_2 or H ions; the center is said to be more irritable. Whether or not this is due to change in concentration of Hions, in the center itself, as suggested by Winterstein, has not been demonstrated. Decreased irritability is indicated after morphine injections when a greater cH is required to stimulate normal respiration. No single explanation is available to cover all conditions which are now described as due to change in irritability of the center though one may imagine that changes in irritability may be in some instances merely expressions of physico-chemical changes in the equilibrium existing between the blood plasma and fluids in the center.

Although the specific stimulus of the respiratory center is chemical, there is also a nervous mechanism regulating respiration which normally comes into play. When the lungs are distended certain sensory nerve endings of the vagus in the lung tissue are stimulated, causing a reflex inhibition of inspiration and onset of expiration. Similarly the collapse of the lungs stimulates other nerve endings bringing about a new inspiration. Thus the rate of respiration is increased above the rate found when the vagi are cut. This nervous regulation of respiration in conjunction with the chemical regulation makes possible very fine adjustments to the needs of the body. When greater ventilation is needed, due, for instance, to increased cH in the blood, it can be most effectively accomplished by an increase in both rate and depth of respiration. The respiratory center, under these circumstances, sends out stronger impulses than usual, increasing the depth of respiration, but tending to cause a decrease in rate. The rate is maintained or even augmented by the reflex action of the vagus.

Regulation of circulation. The importance of the circulation in a study of the acid-base balance in the body is evident, since it is by means of the blood stream that the acid products of metabolism are transported to the lungs and kidneys. Changes in the arterial blood pressure have been studied in attempting to obtain some information concerning the factors influencing the transportation of these materials. Diminished blood pressure and circulatory failure following excessive artificial respiration have been studied by Y. Henderson (1908) and and Y. Henderson and Haggard (1918) and have been attributed to the loss of carbonic acid. More recently the view has been held that the lowering in blood pressure is due to diminished cH of the blood (Y. Henderson, and Haggard, 1920; Haldane, 1922). The recent work of Dale and Evans (1922) demonstrates conclusively that the effect obtained by these investigators must have been due, not to the change

in cH of the blood, but directly to the decrease in H_2CO_3, independent of the cH of the blood. Dale and Evans showed that a change of pH in arterial blood to 8.0 by injecting sodium bicarbonate did not produce any change in arterial pressure, while the removal of H_2CO_3 by excessive ventilation with a similar change of pH did cause an extreme fall in arterial pressure. The pH of both arterial and venous blood changes comparably by these procedures. Their experiments show that the fall of arterial pressure which accompanies excessive artificial ventilation is due to the depression of the vasomotor centers of the bulb and spinal cord by a decrease in the concentration of H_2CO_3 in the blood and not due to the change in cH. If we attempt to apply these conclusions to the smaller variations which may occur in the more normal individual, a complication is encountered similar to that found in the case of the respiratory center, i.e., there seem to be no constant differences in blood pressure between individuals with high and low concentrations of H_2CO_3 in the blood. Other factors seem to overshadow any effects which might be due to these causes.

ELIMINATION OF ACIDS AND BASES BY THE KIDNEY. The influence of urine excretion on the regulation of neutrality in the organism has been analyzed in great detail by L. J. Henderson (1909, 1911) and Henderson and Palmer (1912–15) in a long series of investigations. Their careful analysis of the factors concerned with acid excretion gives us a clear understanding of the fundamentals of this important regulating mechanism.

Practically all of the food materials absorbed from the gastro-intestinal tract must be eliminated in one form or another by way of the lungs or kidneys. In man most of the carbonic acid formed in the body is lost through the lungs. Much of the water is eliminated by way of the lungs and skin. Most of the inorganic salts and organic matter (not CO_2) is excreted by the kidneys together with a considerable amount of water. The kidneys are therefore called upon to regulate not only the osmotic relations within the organism, but also the relative quantities of basic and non-volatile acidic radicles. All of the basic radicles and part of the acidic radicles are taken in as salts in the food while some of the acids, such as sulphuric and phosphoric, are formed from neutral protein material by oxidation in the organism. Some foods contain salts (such as acid potassium citrate) of acids which are oxidized to carbonic acid and thus furnish base to the body (Sherman and Gettler, 1912). The normal food of man contains less basic radicles (such as sodium, potassium, calcium and magnesium) than acidic radicals, if one includes the

phosphorus and sulphur of the proteins which in the body are oxidized to phosphoric and sulphuric acids. In pathological conditions such as diabetes mellitus, large quantities of organic acids may be formed and must be eliminated. Occasionally, to a less extent, due to changes in dietaries or unusual conditions of stress, bases tend to accumulate in the organism. The presence of an effective mechanism for eliminating an excess of bases or acids from the body is therefore necessary. As an excess of acid in the body is more liable to occur, the mechanism for eliminating acid and conserving base is of the greatest practical interest.

Normal human urine ranges in reaction from pH 4.8 to 7.4 (Henderson and Palmer, 1912, 1914; Newburg, Palmer and Henderson, 1913). At these reactions the strong acids, such as sulphuric and phosphoric, cannot be eliminated as such but only in the form of salts. At pH 4.8, 1 mol of sulphuric acid is combined with nearly two mols of base. One mol of phosphoric acid is combined with a little less than 1 mol of base (NaH_2PO_4), whereas in the body where the reaction is more alkaline, the phosphoric acid is combined with 1.8 mols of base. It is evident, then, that in the excretion of 1 mol of phosphoric acid, more than 0.8 mol of base may be retained by the body and serve to neutralize other molecules of acid. If, however, there is an excess of base in the body, it may be excreted as disodium phosphate or bicarbonate and a decrease in the cH of the urine will result.

This limit of reaction of the urine must necessarily limit the efficiency of the kidneys for excreting acids and might easily lead to an excessive loss of base or a piling up of acid in the organism. However, the substitution of ammonia for some of the fixed base occurs and permits the elimination of acids in the form of neutral salts, and a retention of the fixed base by the body. Each mol of ammonia thus represents the saving of one mol of base for the body.

Ammonium ions when present in the blood are toxic so that the normal concentration there is extremely low. It has been taught for years that this small quantity in the blood is maintained by a small portion of the ammonia (produced during the process of deaminization of amino acids) which had not been changed into urea and furnishes ammonia for neutralization of acids. The urinary ammonia has frequently been found to increase at the expense of the urea and the process of urea formation from ammonia seems to be reversible (Barnett and Addis, 1917).

Nash and Benedict (1921, 1922) have recently shown that the ammonia which appears in the urine is formed by the kidneys and not by

the liver. This observation furnishes a much more satisfactory basis for explaining the delicately adjusted mechanism for excreting bases and acids which regulates the acid-base equilibrium of the body. With the formation of ammonia localized in the kidney, the retention of base by substitution of ammonia, and the retention of base during the excretion of acid radicles by variations in cH of the urine may be more closely related. Decrease in the alkali reserves of the body may be explained as due to *1*, inability of the kidney to excrete acids as rapidly as they are formed by eliminating them as such, or as ammonium salts; *2*, the inability of the kidney to excrete acid radicles even though the mechanism for ammonia formation is unimpaired; *3*, the loss of power to form ammonia with the resulting loss of fixed alkali. The necessity for the excretion of an excess of basic radicles occurs less frequently on account of the limits placed by the gastro-intestinal tract, by vomiting and diarrhea, on the introduction of large quantities of salts, and the absence of formation of base (even organic) in the body.

Studies of the influence of acids and alkalis on the excretion of ammonia date back to the classic work of Walter (1877) who found increased excretion of ammonia in dogs after injection of hydrochloric acid. (It is interesting to note in passing that rabbits do not respond in the same way as they have practically no mechanism for forming ammonia.) Marriott and Howland (1918) found that the ingestion of HCl by man gave an increase in ammonia excretion, while large quantities of NaH_2PO_4 did not. The ingestion of $NaHCO_3$ causes the ammonia excretion to diminish (Janney, 1911–12) and after large doses it disappears completely from the urine (Davies, Haldane and Kennaway, 1920).

The reaction of the urine, while an index of the output of acids, does not furnish much evidence concerning the quantity of acid excreted. Such information may be obtained by titration. Many methods of titrating have been used, the most popular being that in which phenolphthalein (end point, pH 8.5) is used as the indicator. L. J. Henderson (1911) emphasized that its use was due mainly to its convenience and suggested as a more scientific procedure, titration to the pH of the blood. Such a titration yields an estimate of the amount of acid excreted over and above the normal reaction of the blood, i.e., the physiological elimination of acids. On account of the buffering of the phosphates, the difference between the two methods of titration may be considerable. As it has been shown that the ammonia of the urine represents acid eliminated from the body, the equivalent amounts of

ammonia and titratable acid may be added together to furnish information concerning the total acid excreted. There is, however, a considerable amount of ammonia in urine, even at pH 7.4 (the normal reaction of the blood) and it disappears only when the reaction becomes more alkaline. Fitz and Van Slyke (1917) suggested, for this reason, that the older method of titrating with phenolphthalein is more satisfactory in that, as the end point more nearly approaches the reaction of the urine obtained when the ammonia excretion is zero, the ratio of ammonia to titrable acid remains more nearly constant. For obtaining an estimate concerning the relative sensitiveness of the two kidney mechanisms, i.e., base retention by ammonia formation, and base retention by alteration in the ratio of basic to acid phosphate, the titration with phenolphthalein would seem to be the more satisfactory.

Under normal conditions, an excess of acid must be excreted by the human kidney if the store of base is to be maintained. This is accomplished by the excretion of urine more acid than the blood and containing a greater ratio of NaH_2PO_4: Na_2HPO_4 than existed in the blood, thereby retaining a portion of the base. Part of the acidic radicles are eliminated combined with ammonia formed in the kidney, with a further retention of fixed base. Gamble (1922) has recently studied the rôle of these mechanisms in retaining base. The three variables, cH, acid titration and ammonia, tend to rise and fall together. As most of the titratable acidity is due to phosphates, it may be said that the kidney makes use of two mechanisms for eliminating acids; the excretion of H_2PO_4 and the formation of ammonia in roughly equivalent amounts, with a greater saving of base to the body the more acid the urine becomes. Actually, the acid-forming mechanism seems to be more sensitive and elastic, as greater changes occur in the acid titration than in the ammonia excretion (L. J. Henderson and Palmer, 1914; Davies, Haldane and Kennaway, 1920).

When abnormal organic acids such as β-oxybutyric acid and aceto-acetic acid are present, the urine may become sufficiently acid so that a considerable proportion of these acids is excreted uncombined with base. Under these conditions another chemical process takes place which is distinctly favorable to the organism. A considerable portion of the aceto-acetic acid formed in the body is decomposed to form carbonic acid (which can be eliminated by the lungs) and the neutral compound acetone, which can be eliminated by the kidneys without loss of any base. It actually happens that a large proportion of the total aceto-acetic acid which is formed in the body and cannot be burned is excreted in a form which requires no loss of base from the body.

Excessive quantities of base can be removed more easily. The limit seems to be merely that $NaHCO_3$ cannot be excreted in a solution more concentrated than 0.3 N, so, with sufficient quantities of water available, large quantities of base may be eliminated. Most of the determinations of the cH of alkaline urines reported in the literature are grossly in error, owing to the loss of free carbonic acid. Marshall (1922) and Gamble (1922) found that even after bicarbonate ingestion the urine never became more alkaline than pH 8.0, the limiting factors apparently being the maximum concentration of bicarbonate in urine and the CO_2 tension in the kidney tissue.

THE REGULATION OF URINE ACIDITY. Although the usual physiological discussion lays emphasis on the fact that the kidneys aid in regulating the acid-base balance of the blood, the problem of greatest chemical interest is just the reverse. How does the acid-base balance of the blood regulate urine excretion? What factors in the blood influence the elimination of each of the acidic and basic radicles from the body?

The problem is of even greater complexity than the similar problem in connection with the regulation of the action of the respiratory center. Whereas the respiratory center is concerned with the elimination and absorption of the gases of the blood, the kidney is concerned with the elimination of solid materials which must be excreted in solutions not too concentrated, leaving the blood with a suitable osmotic pressure. Not only must the total osmotic pressure of the body fluid be kept within normal limits but also the concentration of each constituent in the blood.

Most of the available information indicates that the cH of the urine varies with the cH of the blood. As the urine is normally more acid than the blood plasma, it is similar in this respect to the fluid in the corpuscles but with this striking contrast. Whereas the difference in reaction between corpuscles and plasma diminishes as the blood becomes more acid, until the two fluids have the same reaction at a pH of about 6.5, the difference in reaction between urine and blood plasma increases, reaching a maximum when the urine has a pH of 4.7 and the blood a pH not much less than 7.0. With increasing alkalinity, the two fluids have the same reaction in the neighborhood of pH 7.8.

In general, as the plasma becomes more acid, the cH, acid titration and ammonia in the urine increase. This is shown in experiments involving the administration of acids or morphine or breathing high tensions of CO_2 (for reference, see below). The experiment may be reversed by administration of $NaHCO_3$ or by forced breathing, when the

plasma becomes more alkaline and the cH of the urine, titratable acidity and ammonia decrease. There is, however, at least one interesting exception to this simple control. Several hours after a severe hemorrhage the blood becomes more alkaline than normal while there is an increased excretion of acid and ammonia in the urine, which may persist for several days (Evans, 1921, a; Endres, 1922, and unpublished observations of the author). Evans has described this as a favorable mechanism for maintaining a higher alkalinity in the blood, but such an explanation is hardly helpful in studying the physicochemical mechanism underlying the observation.

Haldane and his co-workers (Davies et al., 1920, 1922; Baird et al., 1923) have reported observations showing a very interesting interrelationship involved in the excretion of the two acidic radicles Cl and HCO_3. They have shown that the sum of the two cannot exceed 0.3 N in the urine and a diminution in the concentration of HCO_3 in the blood is accompanied by a rise in Cl without any tendency toward increased excretion of Cl in the urine.

VARIATIONS IN THE ACID-BASE EQUILIBRIUM. In applying a consideration of the mechanisms involved in neutrality regulations in the body to the everyday life of the individual one is struck by the constant need for such mechanisms. The student of today, to whom the narrow limits of reaction compatible with life have been emphasized so frequently, is liable to exaggerate the narrowness of these limits. On examining the data available at present one is inclined to place the limits of acidity and alkalinity compatible with life at about pH 6.9 to 9.0 and one is surprised to find that these limits are closely approached in some of the ordinary activities of life. It would appear that the factors of safety are not so extensive in this regard as in many functions in the body, but such a conclusion is hardly justified when one considers the numerous mechanisms which may be called into play, the summation of whose activities is more than sufficient to cope with the extremes of acid or alkali excess under normal conditions. Nevertheless the organism may be seriously embarrassed at times by exceeding the ability of these mechanisms to maintain optimum conditions. A brief survey of some of the alterations in the acid-base equilibrium, encountered in activities which may be classed as not unusual, may be of interest, even though much of the data is incomplete.

DAILY VARIATIONS. There is evidence of a slight but definite change in the irritability of the respiratory center at different times of the year as indicated by variations in the alveolar CO_2 of as much as 2 to 5 mm.

Hg (Straub et al., 1914–15 a). Far more striking changes occur during the course of 24 hours. Numerous investigators (Straub et al., 1914–15 a; Leathes, 1919; Endres, 1922) have shown that the alveolar CO_2 tension is greater at night than during the day. The high alveolar CO_2 is associated with a lowered alkali reserve (Collip, 1920) and with the excretion of urine which compared with the day's urine contains more ammonia, a higher titratable acidity and has a higher cH (Leathes, 1919; Campbell and Webster, 1921, 1922 b; Endres, 1922). All of these observations indicate that the cH of the blood is higher at night than during the day due presumably to a decreased irritability of the respiratory center during sleep. Higgins (1914) found that even a change in posture, such as lying down, increased the alveolar CO_2.

Effect of drugs and changes in nervous excitability. Changes in irritability of the respiratory center obtained by the use of drugs produce alterations in the acid-base equilibrium of the body. Morphine, which depresses nervous sensibility, causes a rise of alveolar CO_2 (Straub, et al., 1914–15 b; Endres, 1922) and excretion of urine with a higher cH. Caffein causes a lower alveolar CO_2 and lower cH in the urine, and similar changes in alveolar CO_2 have been observed in individuals who were excited and anxious. Increased or decreased nervous excitability, therefore, seems to be reflected in the respiratory center which responds accordingly and causes the aeration of the blood to be adjusted to a slightly different level of cH, which is maintained in part by the respiratory center and in part by the kidneys.

The "alkaline tide" after meals. During the day, other factors come into play to cause alterations in the acid-base equilibrium which may be of sufficiently short duration to escape notice or render observation difficult. A phenomenon which has been known for many years is the "alkaline tide" in the urine, frequently observed after meals. The cause of the alkaline urine has been assumed to be the relative excess of base in the blood caused by the loss of the hydrochloric acid secreted into the stomach during the course of digestion. The hypothesis and even the observations have been criticised (Hasselbalch, 1912; Leathes, 1919) but the phenomenon has been observed so frequently that there seems no doubt about its occurrence (Campbell and Webster, 1921; Fiske, 1921; Endres, 1922 and Hubbard and Munford, 1922). The careful experiments of Dodds and his collaborators (1921–23) (also Bennet and Dodds, 1921) prove beyond a doubt that the secretion of HCl into the stomach leaves the body tissues with an excess of base, which results in a retention of CO_2 and a rise in bicarbonate of the blood,

an increased alveolar CO_2 (also Higgins, 1914; Straub et al., 1914–15 c; Endres, 1922) and a blood probably slightly more alkaline than normal. The kidneys respond to the decreased cH of the blood by excreting less acid and less ammonia. There is a rise of 2 to 6 mm. in alveolar CO_2 within half or three-quarters of an hour after meals, the height varying with the amount of HCl secreted into the stomach. A subsequent fall occurs to 2 to 6 mm. below the normal level due to the pouring out of alkaline pancreatic juice. These observations were controlled by the introduction of food products directly into the stomach and into the duodenum, and with other experimental procedures.

Gastric tetany. The effect of loss from the blood of HCl secreted in the gastric juice is brought out most strikingly in conditions of gastric obstruction when the gastric juice cannot pass into the duodenum but is vomited. In these cases, the continuous loss of HCl results in a rapid increase in the bicarbonate of the blood, and tetany, which is quickly fatal if the obstruction is complete (McCann, 1918; MacCallum, et al., 1920; Hastings, Murray and Murray, 1921).

Administration of bicarbonate. A similar though less extreme condition results from administration of sodium bicarbonate by mouth or by intravenous injection. There results an increased concentration of bicarbonate in the blood, increased alveolar CO_2 tension, more alkaline blood and the typical response of the kidney: urine less acid and containing less ammonia.

Voluntary overventilation. In the condition of overventilation the picture is rather different (Grant and Goldman, 1920; Collip and Backus, 1920; Davies, Haldane, and Kennaway, 1920; Koehler, 1923). An excess of carbonic acid is driven off from the lungs and the cH of the blood lowered thereby (pH 7.45 changed to pH 7.65 in one experiment). The CO_2 combining power of the blood is not appreciably altered in these experiments (Davies, Haldane and Kennaway, 1920) though, of course, the CO_2 tension is diminished. The kidneys respond by excreting base, the acidity and ammonia excretion diminish and organic acids may appear.

Symptoms of tetany may result from each of the above conditions in which there is but one point in common, namely, blood more alkaline than normal. In reviewing the work on tetany, Greenwald (1922) pointed out that the excess alkalinity of the blood will result in diminishing the dissociation of oxyhemoglobin and thereby tend to induce an oxygen want in the tissues. The muscle tetany and the oxygen want per se may both cause the development of lactic acid which can act as

a protective agent in decreasing the alkalinity of the blood and thereby causing a greater dissociation of oxyhemoglobin.

HIGH TEMPERATURES. An increase in temperature of the individual affects the acid-base equilibrium of the blood in a way very similar to voluntary over-aeration. (Haggard, 1920; Bazett and Haldane, 1921; Koehler, 1923; Pemberton, Crouter and Cajori, unpublished.) An increase in body temperature brought about by hot baths has been shown to cause a stimulation of the respiratory center with a consequent over-ventilation of the lungs and a reduction of the carbonic acid in the blood. The blood becomes more alkaline (the pH may rise 0.10 to 0.20), the alveolar CO_2 and CO_2 content of the blood fall, though the CO_2 absorption curve may rise. The cH of the urine falls and the excretion of ammonia and acids diminishes. Bicarbonate is excreted in the urine and acetone bodies have been found. Not only do these variations appear when the temperature is raised by artificial means but similar changes may ocur in natural fevers. Koehler found in patients with fever the pH of the blood increased 0.10 to 0.15 above the values after recovery.

EXCESS OF ACIDS. When an excess of acids floods the body, due either to acid ingestion or to an increased formation of organic acids just the opposite condition is encountered. The stores of base in the form of bicarbonate are drawn upon and the stronger acid displaces the carbonic acid. The carbonic acid is eliminated by way of the lungs, and the non-volatile acid by the kidneys. Some of the base is conserved for the body by the excretion of a portion of the acid as the ammonium salt and a portion as the free acid. The extreme variations in blood and urine in different individuals depend upon the amount of acid introduced and upon the efficiency of all of the regulating mechanisms.

MUSCLE EXERCISE. Increased oxygen consumption and carbon dioxide formation are the necessary accompaniments of muscle exercise. To supply the increased quantity of oxygen and to facilitate the removal of the increased quantity of carbon dioxide, the nervous, circulatory and respiratory systems act in coördination (Bainbridge, 1919, Douglas and Haldane, 1922) but do not always succeed in maintaining the normal conditions in the body.

Until recently only isolated observations have been made on the changes in arterial and venous blood before and after exercise. The recent experiments of Lundsgaard and Möller (1923 a, b) Barr, Himwich and Green (1923) and Barr and Himwich (1923 a, b) furnish more details concerning the changes which may occur after short periods of strenuous muscular exercise.

During muscular contraction lactic acid is formed, the larger part of which is synthesized to glycogen and the remainder oxidized to carbon dioxide and water. During strenuous muscle activity lactic acid is formed in large quantities and some diffuses out from the muscle along with the increased quantity of carbon dioxide. The lactic acid, reacting with plasma bicarbonate, forms a salt with the base present and an equivalent amount of free carbon dioxide, which, together with the CO_2 entering from the tissues, produces a rapid increase in the carbon dioxide tension of the blood. The increased concentration of H_2CO_3 and decreased concentration of $BHCO_3$ cause an increased cH of the blood. The increased cH of the blood increases the dissociation of oxyhemoglobin (Barcroft, 1914) and favors the passage of more oxygen to the tissues. The loss of oxygen permits the liberation of base from hemoglobin. Thus, the mechanism involving the reciprocal exchange of carbon dioxide and oxygen described as the isohydric change comes into play to an exaggerated degree. But the carbonic and lactic acids from the muscle cannot be completely neutralized by the base released from hemoglobin so the venous blood coming from the muscle has actually a higher cH than normal. During the first minutes of a short period of strenuous muscle exercise the arterial cH and arterial and alveolar CO_2 tensions are all above normal. The increased cH stimulates the respiratory center, and hyperpnea results with a greater aeration of the blood. The aeration is sufficient to cause a fall in the arterial CO_2 tension but the continued passage of lactic acid from the muscle into the blood causes the cH of the blood to rise (the pH may fall to 7.05) and the alkali reserve to diminish. In the resting period after exercise the cH returns to normal in a few minutes but the lactic acid is removed more slowly so that the carbon dioxide tension and carbon dioxide capacity may remain low for a half-hour or more.

To counteract the increasing cH of the blood several mechanisms come into play. Lactic acid is taken up by other tissues in the body. In experiments involving strenuous exercise of the legs Barr and Himwich (1923 a) found that the venous blood coming from the arm contained less lactic acid than the arterial blood going to the arm. The diffusion of lactic acid from the blood releases base so that the carbon dioxide capacity increases and the cH in the venous blood is at times even lower than in the arterial, although both are still above normal. While some lactic acid is being removed from the blood by the inactive tissues some is also being excreted in the urine but the excretion of ammonia and a more acid urine assist in conserving base for the body (Talbert, 1920; Campbell and Webster, 1922 a).

In exercise the temperature of the body is increased (39.0°C. or even higher) which has been shown to be of benefit by making more effective the circulatory and respiratory adjustments during exercise (Bainbridge, 1919). It may possibly be of assistance also in helping to maintain the acid-base balance as it has been shown that a rise of several degrees in body temperature brings about a decreased cH of the blood.

The oxygen content of arterial blood increases during exercise due in part to increased saturation of hemoglobin and in part to an increased oxygen capacity. There is presumably an increased utilization of oxygen in the active muscle but owing to the greatly increased blood flow the oxygen in venous blood is not lowered to values much below normal. The oxygen content of venous blood from inactive muscles is, however, very much reduced due presumably to decreased rate of blood flow.

LOWERED BAROMETRIC PRESSURE. The effect of a rapid decrease in barometric pressure as met with in mountain climbing is said to be identical with over-aeration (Hasselbalch and Lindhard, 1916; Haldane, Kellas and Kennaway, 1919–20). Owing to the lack of oxygen the respiratory center becomes more irritable and excessive ventilation results. Carbonic acid is eliminated more rapidly than it is formed, the CO_2 tension is reduced and a diminished cH of the blood presumably results. The cH of the urine is decreased under these conditions and less acid and ammonia are excreted. This picture, as presented by Haldane, is almost the reverse of that originally held by him which postulated the formation of acid metabolic products which caused a lowering of the alveolar CO_2. It should be noted that the only direct experimental evidence at present of an actual decrease in cH of the blood is the study of the urine.

BLEEDING. The uncertainties of such conclusions may be illustrated by observations of Evans (1921 a) and Endres (1922) which have been substantiated by the author (unpublished), on the effects of bleeding. Loss of blood is usually followed immediately by a decreased alkali reserve of the blood. But the animal soon recovers from the immediate effects of the bleeding and, after some hours, the alkali reserve is found to be above normal. The blood at this time has a lower cH than normal and contains more bicarbonate while the urine excreted contains a higher cH and greater titratable acidity and ammonia. In this instance the changes in the urine would appear to indicate a condition in the blood which is just the opposite of that actually occurring. The introduction of this complication in a condition where oxygen want and overventilation may be postulated must render doubtful the interpretation of similar evidence in other conditions.

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THE INTERNAL SECRETIONS OF THE REPRODUCTIVE ORGANS

F. H. A. MARSHALL

Christ College, Cambridge, England

The secretory activity of the generative glands (testes and ovaries) differs from that of other organs of internal secretion in being in a very marked degree cyclical; that is to say, that although the hormones are probably elaborated to some extent at all times especially during the period of reproductive life which extends in the female from puberty until the climacteric, and in the male from puberty until extreme old age, the secretions change both in composition and in amount with certain recurrent seasons which are correlated with the times for breeding. This periodicity is partly inherent in the reproductive organs themselves, but it is also much influenced by external or environmental factors such as nutrition and seasonal and climatic conditions.

THE OVARIAN SECRETIONS AND THE OESTROUS CYCLE. In the female the recurrent changes in the generative organs and the phenomena associated with them characterise what is known as the oestrous cycle, and this is divided into a number of periods which are as follows:

(1) Anoestrum or period of quiescence.
(2) Prooestrum or period of "coming on heat."
(3) Oestrus or period of desire.
(4) Pregnancy or (alternatively) pseudo-pregnancy.

This is the typical succession in monoestrous animals such as the dog in which there are commonly two sexual seasons in the year and a single oestrus or "heat" period in each sexual season. In polyoestrous animals such as the mare, cow, sheep, pig, rat and mouse, there are several oestrous periods within one sexual season, and these periods are followed by short intervals of comparative quiescence which are known as "dioestrous periods." In such animals pseudo-pregnancy does not usually occur or else is very abbreviated. (The terminology here employed is mainly that originally proposed by Heape.)

The changes which occur during the oestrous cycle relate to the ovaries, the uterus, the vagina and the mammary glands, and there are also general metabolic changes concerning which less is known. The

ovaries contain the controlling factors for all these changes for if these organs are removed the oestrous cycle is brought to an end. If the operation be done prior to puberty the uterus remains infantile and the mammary glands also fail to develop. If the ovaries be removed after puberty when the cycle has started the uterus undergoes atrophy and the mammary glands retrogress unless the operation be done during lactation. On the other hand if the ovaries (or one of them) be transplanted to an abnormal position, notwithstanding the fact that their normal nerve connections are severed, the grafted organ continues to exert its usual influence upon the metabolism, and the oestrous cycle is continued. The assumption is therefore that the ovary exerts its influence upon the other generative organs and upon the metabolism through its internal secretions.

The ovarian elements which seem capable of possessing internal secretory functions are the follicular epithelial cells, the luteal cells of the discharged follicle (these being derived by hypertrophy mainly or entirely from the follicular epithelial cells) and probably also the interstitial cells which are commonly present in the theca interna of the wall of the follicle, in the hilum, and sometimes in the stroma of other parts of the ovary. The interstitial cells constitute the "puberty gland" of Steinach and other authors.

Fraenkel supposed that menstruation in man was brought about through the influence of the corpus luteum. But on the assumption that the menstrual phenomena of the primates are homologous with the prooestrus changes of the lower mammalia, this theory becomes at once untenable, since it has been shown that in the bitch, the sow and various other animals ovulation takes place during oestrus and therefore not until the prooestrum is over; moreover in the bitch the heat periods recur at infrequent intervals (typically once in six months) and a corpus luteum formed from a follicle discharging at one period degenerates before another period is due. It would seem certain therefore that heat in animals and the corresponding processes in man must be brought about by the action of an ovarian secretion arising either in the follicular epithelial cells or in the interstitial cells or possibly in both. In this connection it may be pointed out that according to Lane-Claypon and others the follicular cells and the epithelioid interstitial cells have a common embryonic origin from the primitive germinal epithelium.

Some experiments on the ovaries of bitches (Marshall and Runciman) seemed at first to afford evidence that the presence of mature follicles is not essential for the occurrence of heat. In two animals all the

follicles approaching ripeness, at any rate as far as could be seen, were ruptured artifically by pricking, a few weeks before a heat period was expected, the duration of the oestrous cycle having previously been noted for each individual. Notwithstanding the operation heat occurred in each of the two bitches at about the usual time. The inference at first drawn was that the normal processes of follicular maturation and the phenomenon of heat are both effects of some further factor which must be sought for in the ovaries elsewhere than in the ripe follicle. Interstitial cells are present in the ovaries of the bitch, but they are said to be absent in some mammals (O'Donoghue), and there is no direct evidence that these cells are more active during the heat periods than at other times. Moreover, in two later experiments on bitches, after destroying the mature follicles with a cauterized needle, heat did not supervene at the expected times although the animals had made a complete recovery from the operations, but at a very much later date one of the bitches again came in heat. (Marshall and Wood—unpublished experiments.) Robinson has suggested that in the recorded experiments the cells of the ruptured follicles were not necessarily functionally interfered with notwithstanding the fact that at the time of killing (a short time after heat was over) they had developed into luteal cells. Robinson states further that in the ferret oestrus is experienced only when the follicles are in a certain stage of development which he calls the "pre-inseminal stage." In this animal oestrus may persist for an unusual duration of time in the absence of the male, and during the whole of this period the ovaries contain follicles which remain in the pre-inseminal stage without either discharging or undergoing atrophy. Eventually the follicles with their contained ova degenerate and oestrus passes off. Robinson believes therefore that the mature follicles provide the internal secretion which is responsible for the phenomena of the prooestrum and oestrus. Pugh has recently described what is perhaps a comparable condition in cows and heifers affected with "nymphomania," an abnormal state in which they are continuously "bulling" and always ready to receive the male. Some of the larger follicles become cystic without being septic (as may happen with discharged follicles, which become infected from the Fallopian tubes and uterus) and Pugh suggests that the cystic condition stimulates the growth of the follicular epithelial cells and so favours the production of the internal secretion which is in some way responsible for the continuous oestrus. Lothe also has found that when heat in cows is con-

tinuous or too frequent the condition is very frequently associated with cystic ovaries usually accompanied by endometritis or cervicitis.[1]

The conclusion clearly to be drawn from all these experiments and observations is that the ovarian hormone which produces oestrus or heat is different from that which is responsible for maintaining the normal uterine nutrition.

THE CORRELATION BETWEEN THE CORPUS LUTEUM AND THE UTERUS. The part played by the corpus luteum is much clearer. Broadly speaking this organ is responsible for the changes which take place in the accessory female generative organs and mammary glands during pregnancy and pseudo-pregnancy. We may now briefly consider the evidence for this statement.

In the former times various functions had been assigned to the corpus luteum but the suggestions made were all of them practically devoid of experimental evidence. According to one view it was held that the corpus luteum was the result of excessive vascularization; another theory suggested that the structure was of the nature of a "stop gap" to preserve the cortical circulation of the ovary by preventing an excessive amount of scar tissue; a third theory affirmed that the corpus luteum existed to prevent ovulation during pregnancy. That ovulation does not take place in the presence of a functionally active corpus luteum would seem to be generally true, but it is also true that oestrus does not supervene under such a condition, and it is more in accordance with the facts to regard the suppression of ovulation as of the nature of a consequence rather than a purpose.

Prenant seems to have been the first to suggest that the corpus luteum was a ductless gland which exercised an influence over the general metabolism, but to Fraenkel belongs the credit of assigning to this organ a definite rôle as an internally secreting gland, and basing his theory on definite experimental evidence. According to Fraenkel's theory the corpus luteum possessed the function of elaborating a hormone which in some way assisted in the attachment of the fertilized ovum to the uterine mucous membrane and in the maintenance of its nutrition during the first part of pregnancy. The evidence was derived mainly from the results of experiments in which the ovaries were removed or the

[1] The writer has examined a section, kindly sent him by Mr. Pugh, of an ovary of a nymphomaniac cow and this shows an unusually large number of capillaries running through the stroma. Epithelioid interstitial cells are also present in some number, besides follicular epithelial cells in normal follicles of various stages of growth.

corpora lutea destroyed, and in each case the pregnancy was brought to an end. Control experiments proved that the effects were not merely post-operative. The theory was afterwards extended to explain the cause of menstruation and "heat" and the various phenomena which other investigatiors had attributed to the ovarian secretion, but as already indicated, this view as to the rôle of the corpus luteum cannot be made to apply to the lower mammals with infrequently recurring periods of oestrus. Moreover, although the original part of Fraenkel's theory is now generally accepted, it is not justifiable to regard the corpus luteum as the responsible factor in the attachment and nutrition of the ovum or early embryo in any other sense than that implied in the statement that this organ, through the secretion it produces, acts as a stimulus to the growth of the uterine mucosa and the maintenance of the increased uterine nutrition which are necessary for the occurrence of gestation. Fraenkel's experiments on ovariotomy and the destruction of corpora lutea have been confirmed by numerous other investigators, but it was not until Ancel and Bouin had shown that the corpus luteum exerts a comparable influence on the rabbit's uterus in pseudo-pregnancy that the general conclusions regarding the function of that organ were placed on a completely firm foundation.

As Heape showed long ago, the rabbit normally ovulates only after coition. Consequently the presence of corpora lutea in the ovaries of that animal is usually correlated with the occurrence of pregnancy, and the so-called corpora lutea spuria in the rabbit do not exist. If, however, pregnancy is prevented through coition being made sterile, as by submitting the male to vasectomy, then ovulation takes place and corpora lutea are formed unaccompanied by gestation. Ancel and Bouin were the first to show that in such circumstances the uterus undergoes growth, glandular increase and vascularization followed later by retrogressive changes, and that these changes are clearly correlated with the development and subsequent decline of the corpora lutea in the ovaries. Moreover the uterine changes present a general similarity to those occurring in pregnancy, but decidual tissue is not normally formed.

Hill and O'Donoghue have described what are evidently comparable changes, but occurring normally after oestrus, in the non-pregnant marsupial cat (*Dasyurus viverrinus*). These authors were the first to use the name "pseudo-pregnancy" in this connection, though the term had been previously applied to describe an abnormal condition resembling pregnancy in man, by Matthews Duncan. In the marsupial

cat ovulation takes place spontaneously and there is only one sort of corpus luteum formed irrespectively of whether pregnancy or pseudo-pregnancy supervenes. During pseudo-pregnancy the uteri enlarge and become considerably vascular, and these changes are succeeded by degeneration and desquamation of epithelium with an extravasation of blood in just the same kind of way as happens in the experimentally produced condition of pseudo-pregnancy in the rabbit. Eventually regeneration sets in and the mucous membrane undergoes recuperation.

The dog also has a normal pseudo-pregnant period if ovulation (which in this animal is likewise spontaneous) is not succeeded by true gestation. The development of the uterine glands is very similar to that which takes place during pregnancy, but in the latter condition the secretion formed is a source of nutriment to the fetus. The entire sequence of changes is correlated with the contemporaneous series of ovarian changes, the corpus luteum undergoing a degree of development comparable to what occurs in gestation and persisting for a period nearly or quite as long (Marshall and Halnan).

In the other mammals for which the changes during the oestrous cycle have been described there is either no pseudo-pregnant period or else it is very much abbreviated in correlation with the shortened period of persistence of the corpus luteum which after the dioestrum or short period of sexual rest, makes way, so to speak, for a new heat period and a new ovulation. In the sow Corner has described post-oestrous activity on the part of the uterine glands for eight or nine days, after which the epithelial cells slowly revert to a condition characteristic of heat. The changes therefore are suggestive of a shortened pseudo-pregnancy occurring under the influence of the corpus luteum which undergoes retrogressive changes in the later part of the dioestrum. It is possible also that the growth stages of the menstrual cycle of man represent partly a pseudo-pregnant growth and that the destruction stages similarly are not simply prooestrous but correspond to some extent to pseudo-pregnant regression, owing to the whole cycle of changes being compressed into one month. Lipes states that in man the constructive uterine stage commences as soon as post-oestrous repair is completed, and there is often great glandular development. Moreover, according to Hitschmann and Adler, the premenstrual uterus undergoes changes which are similar in character to those observed in the pregnant uterus, so that it is possible to regard these as taking place under the influence of the corpus luteum.

However this may be, it seems certain that the post-oestrous uterine changes in the non-pregnant maruspial cat, bitch and rabbit (in the latter animal only usually occurring under experimental conditions) are physiologically homologous, and that they take place under the influence of the corpus luteum, since apart from the observed relation between the growth and regression of the corpus luteum on the one hand, and the uterine mucosa on the other, the phenomena of pseudo-pregnancy in the rabbit only supervene after a sterile coition which induces ovulation and admits of the subsequent formation of the corpus luteum.

The existence of a uterine cycle correlated with the ovarian cycle has also been shown in the case of the guinea pig by Leo Loeb.

Decidual cells are not normally formed in the uterine mucosa excepting in true pregnancy, but as was first shown by Loeb in the guinea pig nodules composed of decidual tissue can be induced to develop under the influence of direct stimuli to the mucosa such as the introduction of a foreign body into the uterine cavity or the making of a number of incisions in the mucosa so as to break the continuity of the tissue. The nodules which Loeb describes under the term "deciduomata" arise through the proliferation of the inter-glandular connective tissue. They can be induced to form most readily from the third or fourth to the eighth or ninth days after heat and therefore at a time when freshly formed and active corpora lutea are present in the ovaries. The formation of decidual tissue was not caused by ova in the uterus, since it took place when that organ was ligatured off so as to prevent the passage of the ova. If however the ovaries with their contained corpora lutea are extirpated deciduomata are not produced. On the other hand, if pieces of uterine mucosa are transplanted into subcutaneous tissue decidual nodules are formed in the grafted tissue. Loeb concludes therefore that for a certain interval after ovulation the corpora lutea elaborate a predisposing substance in the presence of which indifferent stimuli may produce the formation of deciduomata.

Hammond has shown further that placental tissue may be formed in the uterine mucosa of the rabbit by similar methods but only during an experimentally induced pseudo-pregnancy. Such a formation of decidual tissue is clearly comparable to that produced during true pregnancy when corpora lutea are normally present in the ovaries.

Long and Evans state that in the rat owing to the short dioestrum and the corresponding abbreviation in the duration of the "corpus luteum spurium" or "corpus luteum of ovulation" deciduomata cannot

be induced in the uterine mucosa. If however the female rat undergoes a sterile coition with a vasectomized male the corpus luteum persists for a longer period and the subsequent oestrus is postponed. This is believed to be due to the formation of the vaginal plug which extends into the cervical canal of the uterus and has a direct stimulating effect on the mucosa, producing a condition of pseudo-pregnancy, and the corpus luteum itself persists for a longer period. The same result can be brought about in the absence of the male by mechanical stimulation of the tissues at the anterior end of the cervix by a tube or glass rod. Further if during pseudo-pregnancy the uterine mucosa were subjected to irritation induced by injury or by the introduction of fine threads into the uterine cavity deciduomata were formed in just the same kind of way as with the guinea pig or the rabbit. It is believed therefore that the internal secretion of the persistent corpus luteum sensitizes the uterine mucous membrane, thereby rendering it capable of reacting to mechanical stimulation in the rat just as it has been shown to do in the other animals experimented upon. In the pregnant animal in which the corpus luteum also persists the direct stimulus is produced by the fertilized ovum.

THE CORRELATION BETWEEN THE CORPUS LUTEUM AND THE MAMMARY GLANDS. That the growth of the mammary glands is dependent upon a stimulus derived from the corpus luteum was first shown by Ancel and Bouin in the rabbit. In the virgin rabbit the mammary tissue is limited to a few ducts in the immediate neighborhood of the nipple. After ovulation however growth proceeds rapidly both in pregnancy and in pseudo-pregnancy and at a certain stage of development of the tissue the hypertrophy is sufficient to allow of secretory activity. As already stated ovulation in the rabbit depends upon coitus, so that the pseudo-pregnant hypertrophy of the mammary glands only takes place ordinarily under experimental conditions such as when a vasectomized buck is used. In the marsupial cat, as shown by O'Donoghue, pseudo-pregnant mammary development followed by milk secretion takes place also, but in this animal ovulation is spontaneous. The bitch is similar. Since however the post-oestrous development of the mammary glands (like the synchronous hypertrophic changes in the uterine mucosa) only takes place in the rabbit in the presence of corpora lutea, and since the parallel series of changes in *Dasyurus* and the dog are also always associated with the development of luteal tissue, there can be no doubt that the corpus luteum is an essential factor for the growth of the milk glands in all these animals. Moreover, the commencement of milk

secretion is marked by retrogressive changes in the corpus luteum. Leo Loeb states that in the guinea pig the corpora lutea are responsible for the secondary growth of the mammary tissue which occurs in the later period of gestation.

In the rat the corpus luteum formed as a result of ovulation occurring immediately after parturition is described by Long and Evans as the "corpus luteum of lactation," and it would appear that in this animal at any rate luteal tissue may undergo development at the same time as actual milk secretion, and it is well known that the same process occurs to a limited extent in such polyoestrous animals as the cow. It would appear possible therefore that the anabolic changes involved in the building up of mammary tissue and the katabolic changes concerned in actual milk secretion may go on simultaneously, and that the corpus luteum may exert an influence on both phenomena which are to be regarded as parts of one process. The fact first discovered by Ott and Scott that luteal extract injected into the circulating blood causes an almost immediately outpouring of milk is evidence supporting this view.

THE CORPUS LUTEUM IN POLYOESTROUS ANIMALS AND THE PERSISTENT CORPUS LUTEUM. It is well known that in polyoestrous animals such as the mare, the ewe, the cow and the sow the corpus luteum persists for only a short time if pregnancy does not supervene after oestrus. This, to speak teleologically, is to admit of heat and ovulation recurring after a short interval, since these processes cannot ordinarily take place in the presence of a fully developed functional corpus luteum in either ovary. It would appear that the corpus luteum when functionally active dominates the ovarian metabolism and inhibits the formation of the internal secretion which is an essential factor in producing prooestrum and oestrus besides hindering the maturation and rupture of the Graafian follicles. In monoestrous animals like the dog, on the other hand, the corpus luteum persists during a pseudo-pregnant period which may be as long in duration as the period of true gestation, since in such animals a new heat period is not due in any case until many months have elapsed after the previous ovulation period. Reference may be made in this connection to the observations of Heape, Noël Paton, Blair Bell and others of non-pregnant bitches, in many cases virgins, secreting milk at about the time when they would have given birth to pups had they become pregnant.

Even in polyoestrous animals, however, provided that ovulation takes place spontaneously in oestrus, the mammary glands may be sufficiently built up during a succession of dioestrous cycles as to admit

of milk formation at a later stage, for Woodman and Hammond, and more recently Asdell (unpublished work), have found that a fluid can be drawn off in some abundance through the teats of virgin heifers, and that the fluid so obtained contains lactose and all the essential constituents of milk. The degree of development and functional capacity of the mammary glands of such animals is in striking contrast to those of the virgin rabbit in which corpora lutea are not formed and in which consequently development of mammary tissue does not take place.

The cat resembles the rabbit in only ovulating after coitus, as shown by Longley, but Doncaster has described a female which after copulating with a sterile tortoiseshell male underwent mammary hypertrophy for about a month and to an extent sufficient to result in secretion of milk which lasted for two weeks. This was clearly a case of pseudo-pregnancy comparable to the condition occurring in the doe rabbit after copulating with a vasectomized buck.

It has been mentioned that in the presence of the corpus luteum ovulation does not take place. In confirmation of this Leo Loeb found in the guinea pig that ovulation rarely occurs within sixteen to eighteen days after a preceding ovulation, but that if the corpora lutea are removed from the ovaries the ovulation interval may be reduced to from twelve to six days. Again, according to Pearl and Surface, the desiccated fat-free extract of cow's corpus luteum when injected into a laying fowl, immediately inhibits ovulation, but after ceasing the injections ovulation and laying proceed as before. Pearl and Surface state that the substance which produces this result is rendered inactive by boiling.

Under certain abnormal conditions the corpus luteum of the non-pregnant cow or heifer may persist for a prolonged period. Such a condition is usually associated with endometritis or some pathological affection of the uterus or Fallopian tubes, and sterility with non-occurrence of oestrus results. That the non-occurrence of oestrus is due to the persistent corpus luteum is proved by the observations and experiments of Zschokke, Williams, Hess, Oppermann, Pugh, Lothe and others who have shown that if the corpus luteum be removed or destroyed oestrus will generally recur within from 3 to 8 days, and the animal may be got to breed. The results of these and other experiments carried out upon cows in veterinary practice are thus in strict conformity with what is known concerning the influence of the corpus luteum in the other animals in which the ovarian and correlative processes have been investigated and described.

THE OVARIES AND PARTURITION. The study of pseudo-pregnancy in the bitch, the experimental rabbit and the marsupial cat throws some light on the factors responsible for parturition. In each of these species there is present at the end of pseudo-pregnancy a persistent corpus luteum in a condition of involution not dissimilar to that of the corpus luteum vèrum at the end of true pregnancy. Further, pseudo-pregnancy can only occur when a corpus luteum is present. Moreover, all these animals display habits and instincts at the end of pseudo-pregnancy which are identical with or similar to those associated with parturition. Thus the bitch may prepare a bed as if for a litter of pups, the doe rabbit plucks her breast of fur and uses it to line a nest (Hammond), and the female marsupial cat cleans out her pouch as though for the reception of young (Hill and O'Donoghue). It has been shown that the occurrence and duration of pseudo-pregnancy are dependent on the corpus luteum, and consequently it is exceedingly probable that the processes associated with parturition are similarly correlated with changes in the amount or character of the ovarian secretions. Ancel and Bouin suggested that whereas in the first part of pregnancy the tolerance of the uterus for the fetus was due to the corpus, during the second part it was brought about through the "myometrial gland" of the uterus, but Hammond has shown that it is far more likely that the corpus luteum is the responsible organ throughout the whole of gestation, since this organ persists and is apparently functionally active until quite a late stage in pregnancy, whereas in pseudo-pregnancy the corpus luteum and mammary glands are never so completely developed and the former organ does not retain its state of maximum growth so long. Furthermore Sharpey Schafer has shown that the ovaries may produce at least two kinds of hormones which act differently on plain muscle, one increasing contractility and the other acting as an inhibitor. The respective amounts of these secretions probably vary at different stages of the cycle and may show a relation to different phases in the history of the follicle and corpus luteum, but further evidence is needed before such a theory can become anything more than a suggestion. Very recently Dixon has carried out some experiments which are calculated to throw further light upon this question. It is well known that pituitary extract promotes uterine contraction, and Dixon has shown that ovarian extract (without corpus luteum) when injected into the circulation causes pituitary secretion, but that corpus luteum extract has no such effect. It is possible therefore that during pregnancy when the corpus luteum dominates the ovarian metabolism, the activity of the normal

ovarian secretion is reduced, but that at the end of pregnancy when the corpus luteum has entered into a state of involution, the ovarian secretion reasserts its influence, and by activating the pituitary promotes those uterine contractions which are the cause of parturition.

THE CORRELATION BETWEEN THE OVARIES AND THE SEXUAL CHARACTERS. As already mentioned, there is an undoubted functional correlation between the ovaries and the normal nutritional condition of the uterus since ovariotomy is followed by uterine degeneration which may be arrested by the successful transplantation of an ovarian graft in an abnormal position. There is some evidence that the ovarian interstitial cells are responsible for elaborating the internal secretion which is responsible for maintaining the normal nutrition of the uterus, for McIlroy found that this is preserved by ovarian grafts in which the follicle cells have degenerated and, of the possible secretory elements, only the interstitial cells remain.

The study of the distribution and comparative physiology of the ovarian interstitial tissue is still however very imperfect and in some animals interstitial cells have not been discovered, at any rate in the ovaries of the adult. Thus, according to Aime, ovarian interstitial cells have not been seen in the sheep, pig, dog or man or in the adult horse, whereas they are stated to be present in the adults of bats, insectivora and rodents, in both the fetal and the adult cat, and in the fetal horse. Robinson states that he has seen them in the dog. O'Donoghue has observed them in marsupials; and van der Stricht and Athias in bats. Cese-Bianchi and Rasmussen have described them as undergoing cyclical changes in hibernating animals such as the woodchuck, becoming most active during the times of sexual activity and becoming much reduced during the winter sleep. Regaud and Dubreuil and Wallart state that the interstitial cells increase during pregnancy. Evans, referring to the rat, says that there is no hypertrophy of the interstitial tissue at puberty. It is not unlikely that there is considerable variation among the different species of mammals in regard to the development and functional importance of these cells, and it must be remembered that according to Lane-Claypon and McIlroy the follicular epithelial cells and interstitial cells have an identical origin and are therefore probably potentially equivalent.

According to Steinach and his followers, the interstitial cells of the ovary represent the "puberty gland" of the female organism, that is to say, that gland which is responsible for all the essentially female characteristics including not only the accessory generative organs such as the

uterus and mammary glands but also the secondary characters of sex and the psychological female characters. The organ is called the "puberty gland" because those characters which depend upon its activity undergo marked development at puberty.

This view is based upon a large number of experiments and observations upon various species of mammals. Male animals are described as becoming "feminized" by the introduction of transplanted ovaries after the previous removal of the testes. Guinea pigs operated upon and treated in this way are stated to have undergone remarkable development of the mammary glands and teats, and to have produced milk and even to have suckled the young of other individuals. The hair of these feminized animals is said to have resembled that of normal females, being finer and softer than in the male. Moreover, the guinea pigs with successfully transplanted ovaries showed typical female reactions, such as the "tail-erect reflex" normally concerned in coitus, and the "kick-guarding reflex" which is employed by the female to ward off the male prior to the onset of oestrus. Athias, Moore and Sand have also described development of mammary tissue and milk secretion in animals (rats and guinea pigs) after the introduction of ovarian grafts.

Steinach and Holzknecht have studied the effects of Röntgen ray treatment on the ovaries. They state that these showed a degeneration of follicles but a survival or even accentuation of interstitial cell growth. Moreover, the increase in the interstitial tissue following a correct x-ray dosage resulted in mammary growth and eventually in milk secretion. In women one of the effects was a postponement of the climacteric. All these results are attributed to the activity of the female puberty gland which is supposed to preside over the metabolism of the accessory sexual organs and the secondary female characters. It has been shown however (as described above) that mammary development is normally due to the activity of the corpus luteum, and if the interstitial cells have any influence over the milk glands in feminized animals it is perhaps comparable to the slight hypertrophy which takes place during the prooestrum before ovulation or possibly to the primary development of the gland at the beginning of pregnancy which, in the guinea pig, Leo Loeb distinguishes from the more pronounced secondary hypertrophy occurring later under the influence of the corpus luteum. Otherwise one must assume that under certain conditions the interstitial cells act vicariously for the corpus luteum.

The experiments by Steinach and others on the masculinization of females are referred to below in dealing with the male "puberty gland."

That the ovaries are functionally correlated with the development of the secondary female characters is more apparent in birds than in mammals, since in birds the neutral type is outwardly much nearer to the male than to the female, and there is little or no evidence that ovariotomy in any animals results in the development of distinctively male characters. Tandler and Keller state that removal of the ovaries in heifers produces a type similar to that of the castrated male, the head resembling the head of the steer. In Herdwick sheep which are horned in the male and hornless in the female, ovariotomy is not followed by the growth of horns (Marshall). In other species of mammals the operation is negative in its effect upon the bodily conformation and appearance.

In birds, on the other hand, ovariotomy is followed by very pronounced results. Goodale and Pézard have shown that in fowls it produces birds similar or identical with castrated males. Thus with Brown Leghorns the spayed hen assumes the plumage of the Leghorn cock, with red back, black breast and long pointed hackle and saddle feathers, and spurs develop on the legs. The sickle and other characteristically male feathers, however, did not appear, and the comb and erectile structures did not hypertrophy as they do in the male. Goodale also removed the ovary from the Rouen duck and found that the bird to a very great extent assumed the plumage of the drake. Duerden and Fitzsimons record that ovariotomy in the ostrich is followed by retention of the ordinary body color, but that the normally grey feathers assume the black coloration of the cock, a fact which has been taken advantage of for commercial purposes by South African ostrich farmers who make a practice of spaying certain of the hens. Zawadowsky has described the effect of ovarian ablation in pheasants, and these are essentially similar to the de-sexed females presenting a close superficial resemblance to cock birds of the same species.

Such observations as these have led Lipschütz and Pézard to elaborate the idea of a neutral or indifferent type upon which the internal secretions of the generative glands operate, and it is suggested that even in the embryo there are sexual endocrine organs which are responsible for initiating the growth of the distinctively male or female characteristics. This idea receives support from Minoura's experiments on chicks. This investigator removed portions of the egg shells during the second week of incubation and transplanted onto the chorio-allantoic membrane portions of gonads from other individuals, and by this method succeeded in producing different grades of intersexuality. The grafts

were obtained both from other chicks and from older birds but the effects were the same, and in female-type embryos the right ovary, which usually atrophies with birds, might be got to persist as a result of a successful ovarian graft. Further experiments dealing with this subject are described below in considering the internal secretory activities of the testis.

It is not known what precise ovarian elements are responsible for the production of the hormone which is an excitant for the development of the female organs and characters or the possible inhibition of the distinctively male ones, and in birds there is even less evidence upon this question than there is in mammals (cf. Hartman and Hamilton).

THE INTERNAL TESTICULAR SECRETION AND THE MALE GENERATIVE CYCLE. In most male mammals there is a rutting season which occurs at the same time as the sexual season in the female, and spermotogenesis is confined to this time. The periodic activity of the testis is usually correlated with a great increase in the size of that organ. Thus in the mole according to Régaud and Lécaillon the testicles increase in bulk sixty-four times.

As is well known, the growth of the accessory male organs and the secondary male characters is dependent upon the presence of the testes, and this is true not only of the pubertal growth but of the periodic or seasonal development where such occurs. Thus the vesiculae seminales, the prostate and other accessory sexual organs of the adult male hedgehog which undergo an enormous development periodically with the approach of rut, fail to do so after the removal of the testes (Marshall), and it is well known that the antlers of the stag do not undergo their annual growth if the animal be previously castrated, notwithstanding that the stag may have reached maturity and grown antlers in the previous year before the operation was performed. Again in Herdwick rams the presence of the testes is not only necessary for the initiation of horn growth but also for its continuance, since the the horns cease to grow forthwith after castration and at any stage of development (Marshall). That the testis exerts its influence through the medium of the circulating blood and therefore by means of an internal secretion is proved by the effect of testicular grafts in abnormal positions in just the same kind of way as with the female. This was first definitely shown by Berthold in 1849.

The glandular substance of the testis consists of the spermatogenetic tissue contained within the seminiferous tubules (together with the cells of Sertoli) which are generally believed to have a nutritive or supporting

function connected with the production of the reproductive cells, and the interstitial tissue consisting of the cells of Leydig which lie outside of and between the tubules and are collectively included by Steinach under the term "puberty gland." They are believed by this physiologist to correspond in the male to the interstitial or puberty gland of the ovary. There has been a considerable amount of controversy as to the seat of production of the internal testicular secretion, but the bulk of the evidence seems to show that it is produced exclusively by the interstitial cells, at least among mammals; indeed the evidence is far clearer in the case of the male than it is for the female.

Bouin and Ancel appear to have been the first to show that when in the horse and other animals the vasa deferentia are ligatured the spermatogenetic tissue of the testis gradually ceases to be functional and eventually undergoes degeneration. This result is possibly due to the semen failing to escape and so exercising an inhibitory influence by back pressure upon the tubules where sperm production consequently ceases. The interstitial cells however, since they are outside the tubules, do not degenerate. Copeman working upon rats obtained similar results. Moreover Shattock and Seligmann found that in Herdwick rams, notwithstanding the degeneration of the spermatogenetic tissue brought about by occluding the vasa deferentia the horns which, in this breed characterize the male, grew normally. Subsequently Tandler and Gross described the effects of subjecting the testes of the roebuck to the Röntgen rays. They found that the spermatozoa and spermatogenetic tissues are destroyed but the interstitial tissue remains unaffected, and in correlation with its presence the horns of the roebuck undergo development as in the normal male. Evidence pointing in the same direction is furnished by those cases of cryptorchism where the undescended testicles come to consist of interstitial tissue only and yet sexual desire is manifested and the secondary male characters are developed. Lipschütz has shown that in the guinea pig a portion of testicular tissue one-sixteenth of the normal size and consisting mainly of interstitial tissue may suffice to admit of the development of the secondary sexual characters. The evidence that the interstitial cells are functionally responsible for the production of the testicular hormone, and that the germinative cells play no part in the process, at least in mammals, seems to be very strong.

That there is an interstitial gland before birth was shown in the horse by Bouin and Ancel who state that it diminishes in the later stages of intra-uterine life, the formation of new interstitial tissue beginning

some time after birth, but not being completed until spermatogenesis occurs. Aron has recently recorded similar observations in the sheep and pig. Lipschütz and others have suggested that this fetal interstitial gland is responsible for the early sexual differences, a conclusion which receives some support from Lillie's work on the free-martin and Minoura's transplantation experiments with chicks. Furthermore Lillie states that Bascom, working in Lillie's laboratory, has found interstitial cells of the same character as those of the adult and filled with secretory granules in the fetal testis of the bull-calf at a stage of development represented by an embryo length of about thirty millimeters.

It has already been mentioned that in the hedgehog the accessory male organs undergo a very pronounced periodic growth after hibernation and with the approach of the breeding season. This is associated with a great testicular development which affects not only the spermatogenetic tissue but also the interstitial cells; indeed the latter proliferate even more than the spermatogenetic cells and the tubules come to be widely separated. Similar facts have been recorded for the mole (Lécaillon) and the woodchuck (Hansemann, Rasmussen).

The remarkable periodic increase in the size of the testis of birds has long been known but the question as to the seat of production of the internal testicular secretion in birds is still an open one. Des Cilleul stated that the appearance of the interstitial cells in the cock coincided with that of the secondary sexual characters, and that the development proceeded synchronously, but other observers have taken a different view. The divergence appears to relate mainly to the question as to whether the so-called interstitial cells in birds are really internally secretory in the sense in which they appear to be in mammals. Thus Boring states that interstitial tissue is abundant in the testes of newly hatched chicks but that there is no evidence that it produces an internal secretion. Reeves found interstitial tissue in young cocks five and one-half, nine and eighteen months old. Pézard maintains that the internal testicular secretion is produced by the germinative cells or the cells of Sertoli. On the other hand Watson found in the greenfinch that there were definite epitheloid interstitial cells but that they showed their most pronounced development in the non-sexual season, and that as spermatogenesis approached they decreased in number. More recently Massaglia, as a result of experiments on the effects of ligation of the vasa deferentia, found that there was a degeneration of the spermatogenetic tissue but no such effect upon the interstitial cells,

and that in correlation with this the secondary male characters were normal. These experiments, therefore, were similar both in method and result to those with the mammals described above. Nevertheless, Stieve working on the jackdaw states that there is no increase in the interstitial cell growth in correlation with the development of the male characters, and is disposed to deny any endocrine function to the interstitial tissue of the testis.

Among the lower vertebrates Aron has adduced evidence of a correlation between the periodic changes of a peculiar testicular gland and the recurrence of oestrus in the newt (*Molge cristata*). The gland is situated near the hilum of the testis; it develops at the commencement of the sexual season when its cells proliferate, their protoplasm becoming filled with large fatty granules; it persists until the time when the nuptial characteristics begin to disappear when the gland also undergoes retrogression. It had already been demonstrated that after castration the male nuptial characters do not make their appearance but Aron found that the same result could be brought about by the destruction of the special gland above referred to at the beginning of heat, notwithstanding the fact that the seminiferous tissue of the testis was not interfered with. If the gland was only incompletely destroyed then the nuptial changes proceeded normally. Aron's conclusions have been criticized by Champy, but there nevertheless appears to be a strong presumptive case that the gland in question is physiologically homologous with the interstitial gland of mammals.

Among fishes it has been shown by Courrier that in *Gasterosteus aculeatus* the testicular interstitial cells assume a glandular appearance at about the time of the nuptial transformation and after spermatogenesis has taken place. In the absence of this change on the part of the interstitial gland the typical development of nuptial characteristics does not occur. Moreover Courrier concludes that the interstitial gland cannot be of the nature of a trophic organ for the spermatogenetic cells, since these undergo development before the interstitial transformation. The general conclusion reached is that the interstitial gland in fishes is homologous with that described by Aron for amphibians and with the mammalian interstitial gland of Ancel and Bouin.

THE TESTICULAR HORMONE CONSIDERED IN RELATION TO SEX DIFFERENCES. It will have been seen that there is strong evidence that the interstitial tissue of the testis produces an internal secretion which is responsible for the developmental changes which occur in the male at puberty as well as for the periodic changes associated with the male

generative cycle, including both those which relate to the accessory generative organs and those shown by the secondary sexual characters. Steinach and his co-workers have gone further and, as a result of a large number of experiments, mostly on testicular transplantations into females, ascribe to the interstitial gland a definite sex-determining influence.

In experiments upon guinea pigs and rats it was found that when the testes were transplanted into young females from which the ovaries had been removed, the clitoris developed into a penile structure and there were manifestations of sexual desire as displayed by males; moreover the animals grew to the size of males. Such individuals are regarded as being sexually inverted or masculinized in just the same way as in the converse experiments males with ovarian grafts apparently became feminized. In such animals only the interstitial gland survived. Lipschütz has studied the anatomy of the hypertrophied clitoris in the inverted guinea pig and found that it contained corpora cavernosa as well as the quill-shaped horny spikes which are characteristic of the penis in that animal. Steinach's experiments have been confirmed and extended by Sand and by Moore who however state that they were able also to produce experimental hermaphrodites, testes being transplanted successfully into entire females and ovaries into entire males when the gonads of each kind exerted an influence on the appropriate accessory sex organs without causing a degeneration of the organs correlated with the opposite sex.

Steinach's theory that the internal secretions of the gonads and probably of the interstitial gland are the principal factor in sex determination, receives strong support from the observations made by Tandler and Keller, and by Lillie, on the "free-martin." These investigators showed independently but about the same time that the bovine free-martin is a partial hermaphrodite or rather an inter-sexual individual which started as a female but became modified during embryonic development. They observed that in such an animal the blood vessels of the chorion anastomosed with those of its fellow, that is, with the vessels of the co-twin which after birth becomes a normal fertile bull. The generative organs of the male were shown to be in a more advanced stage of development than those of the female at the same age, and the conclusion was drawn that the hormone produced by the fetal testis exerted an influence over the organs of the originally female twin at an early stage before the ovary as an internally secreting organ was functionally active. The testicular hormone coming from

the normal male twin was of course carried in the common circulating fluid through the united vessels of the two chorionic membranes. Furthermore, as already mentioned, Bascom has found a well-developed interstitial gland in the testis of the bull embryo at the stage in question. Lastly, Lillie found that the two embryos (free-martin and normal male) arise from different ova as proved by the presence of two corpora lutea in the ovaries, and not from a single ovum as had been previously suggested by Berry Hart and others.

This theory of the free-martin has received confirmation from Minoura's experiments upon transplanting gonads of chicks. As related above, Minoura removed a portion of the shell of the incubating egg and grafted on to the chorio-allantoic membrane a piece of gonad from an individual of the opposite sex, and in this way obtained artificial free-martins or inter-sexual individuals among fowls. All degrees of inter-sexuality were produced. Inter-sexes among pigeons obtained by injecting extracts of gonad as well as by other methods have been described by Riddle. The whole of this work together with many other instances of inter-sexuality among various groups and species of animals is well reviewed by Goldschmidt in his recent work on sex-determination.

The effects of gonadectomy in various vertebrates are also in general accord with Steinach's views on sex-determination. This is seen very strikingly in birds in which it has been shown by Goodale, Pézard, Zawadowsky and others that the result of castration in either sex is to produce a neutral type which in this class is much closer to the normal male than to the normal female, the exact converse being the case in mammals. Thus, removal of the testis in the cock has little effect upon the general plumage or the spurs but the comb and other erectile structures do not develop. In pheasants the results are similar. Ovariotomy on the other hand, as already mentioned, is followed by an assumption of the plumage usually associated with the male and by a growth of the spurs, but the erectile organs do not hypertrophy. Similarly in the ostrich Duerden has shown that gonadectomy in either sex results in similar individuals. Among mammals also Tandler and Keller state that removal of the gonads from cattle produces a convergence of type, this being seen especially in the shape of the head. All these observations are an indication that the gonads play a very important rôle, if not indeed the main one, in determining which sex is to develop, thus confirming the view that under certain conditions the chromosome constitution normally associated with a particular sex can be overridden and the opposite sex produced.

THE "PUBERTY GLAND" AND REJUVENESCENCE. The idea of a connection between testicular influence and rejuvenescence, originally put forward by Brown Séquard as part of his general theory of the metabolic effects of the internal secretions of the gonads, has been brought into prominence again recently by Steinach and some other writers with special reference to the interstitial or puberty gland. Thus it has been claimed by Voronoff that successful transplantation of testicular interstitial tissue into the aged has been followed by rejuvenation and generally beneficial results, and the grafting of interstitial gland obtained from apes has been recommended. Voronoff records further a large series of experiments upon goats, sheep and other animals. Successful testicular transplantation in man had been previously recorded by Steinach, Lichtenstern, and others.

According to Steinach, rejuvenation can also be brought about by vasectomy or ligaturing of the vas deferens, operations which result sooner or later in the atrophy of the spermatogenetic tissue without interfering with the interstitial tissue. It is not clearly understood why the spermatogenetic tissue should be destroyed as a consequence of the operation, and the results obtained by various observers are by no means uniform. Thus in testicular grafts in fowls spermatogenesis is known to continue for some time at any rate after the transplanted organ has become attached and without there being any exit for the seminiferous fluid or spermatozoa. There can be no doubt however that vasectomy does frequently, if not usually, result in cessation in the production of the spermatozoa and sooner or later in the degeneration of the seminiferous tubules as shown originally by Ancel and Bouin. (Cf. Myers, etc.) Steinach goes further and states that as a consequence of the changed conditions in the testis the interstitial tissue undergoes hypertrophy and the resulting rejuvenation of the organism is attributed to this hypertrophy on the part of the puberty gland. The operation has been done upon aged men, both bilaterally and unilaterally, and is stated to have been followed by favorable results. The advantage of the unilateral operation is that one testis remains fully functional.

Steinach found that normal unoperated rats seldom lived for more than twenty-six months but that individuals in which the vas was cut or ligatured might live for thirty-six months, and this increase in the longevity is attributed to the hypertrophy of the puberty gland. It must be pointed out, however, that Slonaker in an investigation upon the natural life of the rat found that the normal duration was about

forty months and Donaldson says that the average duration is only slightly less.

Romeis states that he could find no histological evidence that ligature of the vas brought about any augmentation in the interstitial cells of the testis, and that the increase in size on the part of the vesiculae seminales and prostate which occurred (as in Steinach's experiments) was due to post-operative stasis and was not a true functional hypertrophy.

On the other hand, in confirmation of Steinach, Sand has recorded an experiment upon a dog which showed all the signs of senility but after the operation of bilateral vasectomy was restored to a condition of physical robustness and generally rejuvenated. Harms also has described experiments upon other animals with similar successful results.

A possible fallacy underlying all these experiments is that the result of any nutritional or other environmental influence is not easy to determine and may be neglected while effective control experiments are a matter of difficulty. Moreover, in the case of operated men it is always possible that the results are in part at least due to suggestion.

Mottram and Cramer have recently carried out a series of experiments upon rats in which the testes were irradiated over long periods by successive small doses. They found that the animals put on weight more rapidly than the controls and became very obese whereas castrated rats did not put on fat. The seminiferous tubules underwent intense atrophy and apparently as a result of reduced pressure which admitted of a freer blood supply the interstitial cells became hypertrophied. As a consequence changes (atrophic in their essential nature) were produced in the intermediate and posterior portions of the pituitary. The authors put forward the view that in dystrophia adiposogenitalis where the primary lesion is in the pituitary, the sequelae are first, atrophy of the seminiferous tubules, secondly, hypertrophy of the interstitial tissue, and thirdly, adiposity. It is suggested further that deposition of fat occurring in a senile organism as a consequence of interstitial hypertrophy may be the real basis for the claims of rejuvenation made by Steinach and others in the experiments and observations recorded above. Cramer and Mottram however found that vasectomy in rats was not followed by degeneration of the seminiferous tubules or hypertrophy of the interstitial tissue.

The question, therefore, as to the supposed rejuvenating influence of vasectomy and the hypertrophy of the interstitial gland is still an open one, but there can be no doubt that in mammals the secretion

elaborated by this organ is responsible for the development of the pubertal characters, while there is accumulating evidence that the predecessor to this gland in fetal life is a very important factor in sexual differentiation before birth.

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CHEMOTHERAPY

H. H. DALE

National Institute for Medical Research, Hampstead, London

I. INTRODUCTION. By intoducing the name "Chemotherapy" Ehrlich intended to mark off, from the general body of Pharmacology, a particular type of investigation, having as its aim the discovery of chemical substances acting specifically on pathogenic infections. This attempt to erect a new boundary fence, to delimit a new plot in the scientific field, has not passed altogether without criticism. It could be urged, and not without some justice, that the application of specific remedies to combat particular infections was as old as the use of cinchona for the cure of malarial fevers, of ipecacuanha for that of tropical dysentery, and of mercury for that of syphilis; and that the study of the action of these remedies had long been included in the orthodox pharmacology. It can even today be truly stated that, with the exact knowledge now available concerning the protozoal parasites of malaria and dysentery, chemotherapy has made no real advance on the traditional remedies for these diseases. Such improvements as have been made have resulted from a more accurate knowledge and complete separation of their alkaloids—a type of investigation having certainly a closer relation to the conventional pharmacology than to the special methods of chemotherapy. On the other hand there was justification for Ehrlich's suggestion that pharmacology had devoted attention almost exclusively to a detailed analysis of the symptoms produced in higher animals by toxic doses, even of remedies, such as quinine, which owed their therapeutic reputation to specific cure of definite infections. Chemotherapy, as he conceived it, was to shift the focus of interest to the action on the parasites. Its aim must be the discovery of substances maximally toxic for the infecting parasite, and minimally toxic for the infected host. His lifelong study of the distribution of chemical substances, especially of dyestuffs, among the organs of the body into which they had been introduced, and the chemical terms in which he was accustomed to interpret such observations, provided a conception, diagrammatic in its simplicity, for the

mode of action of such substances. The substance, given the requisite toxic properties, would kill or injure only the cells to which it became fixed by reason of its chemical affinities. The aim of chemotherapeutic investigation, therefore, must be to find toxic substances which, having a strong affinity for the protoplasm of the parasite and a weak affinity for that of the cells of the host, could be administered in sufficient doses to kill the infecting organism and leave the host unscathed. The search must be for substances which are maximally "parisitotropic" and minimally "organotropic."

It will be found difficult, as yet, to form a just opinion as to the rôle which this simple conception has played in the development of chemotherapy during recent years. It is hardly likely that it will retain permanent status as an exact scientific theory. The knowledge yet available concerning the chemistry of the protoplasm has no point of contact with a conception of this kind. Such knowledge affords no suggestion as to the nature of the chemical differences between the protoplasm of the vertebrate and that of the unicellular parasite, and furnishes no basis for prediction, or even for surmise, with regard to their differential affinities for chemical substances of known constitution. When a certain substance is found to cause the disappearance of, say, a particular species of trypanosome from the blood of an infected mouse, without harming the mouse, and fails to remove a similar infection from the rat, when administered in doses which that animal can tolerate, these observations are taken to indicate that the ratio of its parasitotropic to its organotropic properties is more favorable in the mouse than in the rat. Essentially, however, this is a mere restatement of the observed fact, that the mouse can be cured but the rat cannot. The supposition that the result is determined by the distribution of the substance between the cells of the host and the parasites is not based on independent evidence; such evidence as exists, apart from the curative result, is, in fact, not favorable to the conception. For example, the presence in the host's blood of parasites having a preferential affinity for the toxic drug might be expected to exert an antitoxic action, by diverting its action from the tissues. Certainly if this relation were found to be the rule, it would be claimed as evidence in favor of the conception. The relation actually observed, however, is the opposite of this; it is the infected animal which is the less resistant. In animals of the same species, again, suffering from the same infection, the effect of a drug on the parasites should, on the simple distribution theory, become stronger in proportion as its action

on the host becomes weaker. In some cases where a relation between the intensities of the two effects, on the infecting parasite and on the host, has been observed, it is the reverse of this; the more sensitive individual is the more easily cured. Again, the observation that, in many cases, a remedy acting potently against a certain infection *in vivo* is practically without visible action on the parasites *in vitro*, is not, by itself, consistent with the simple conception that its curative action is due to its chemical affinity for the parasites. It is true that these various anomalies can be, and have been more or less successfully reconciled with the original theory attributing curative action to specific chemical affinity; but this can only be effected by the introduction of subsidiary hypotheses, which mostly involve considerations of the interaction between the drug and the tissues of the host. The original conception can thus be redeemed from inconsistency with the observed facts; but the possibility only suffices to show that it is not necessarily untrue. Positive, independent support for the basic assumption of preferential affinity is curiously difficult to find.

In these circumstances it is impossible to feel confidence as to the general and permanent validity of Ehrlich's method of interpreting the facts. On the other hand it cannot be doubted that his bold conception has played a part of enormous importance in stimulating investigation. If the truth of a theory could be judged by the practical results which have resulted from efforts to test and apply it, this one would indeed be firmly established. But though, in the process of opening up a new territory for research, the practical value of a theory may largely be determined by its power of stimulating and encouraging experiment, and even of ignoring difficulties which a more fundamental consideration of the problem might present as insuperable, there comes a time when it is necessary to enquire whether it has not served its purpose. In the case under consideration, Ehrlich's theory has tended to focus attention on the effect of a remedy on the parasite. It led undoubtedly to the exploitation of new methods, which made a much needed departure from those of pharmacological orthodoxy. But there are growing indications that the attempt to interpret the experimental results too exclusively in terms of direct parasiticidal action may eventually hamper progress, by throwing back investigation into an empiricism from which it was Ehrlich's aim to rescue it. The object of this review is to survey the position as it presents itself to the writer at the moment, and to consider, in particular, certain facts which are difficult to reconcile with the simple distribution hypothesis. No

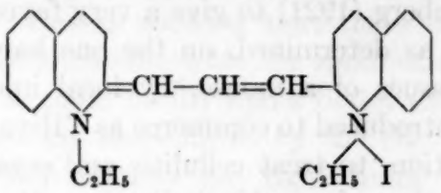
attempt will be made to mention, even by reference, the whole of the enormous wealth of detailed evidence. The aim will rather be so to present the salient and significant points as to show what part of the evidence is in conformity with the idea of direct and simple chemical action, and what part of it, on the other hand, indicates actions of much greater complexity, involving factors for the investigation of which the methods, even, are not yet available.

II. CHEMOTHERAPY OF BACTERIAL INFECTION. The attempts to combat bacterial infections by means of artificial chemical substances have been fewer in number and, on the whole, less fruitful of practical result than those directed to infections with animal parasites. Such as they are, the results are simpler and more easily interpreted in terms of direct action on the parasites. Bechhold and Ehrlich (1906, 1907), prepared a large series of phenol derivatives, introducing halogens and uniting phenolic groupings by bridges of various types, and in this way obtained compounds which far exceeded all previously known organic disinfectants in their lethal action on diphtheria bacilli growing in nutritive bouillon. The experiments were later extended to other bacilli and cocci. In therapeutic experiments, however, conducted on infected animals, none of these substances proved successful. The reason of their failure was more obvious when it was found that their disinfecting potency was enormously reduced by the presence of protein in the medium, as when the organisms were suspended for the test in blood serum instead of broth. Bechhold subsequently showed, by ultrafiltration, that the disinfectant had entered into combination with the proteins of the suspending medium, and had thus been prevented from reaching the bacteria. The observation is chiefly interesting, from its obvious significance in connection with the development of the theory of chemotherapeutic action. Further investigations by Bechhold (1909), concerned solely with disinfection outside the body, are of interest as showing a partial specificity of certain derivatives for certain organisms; thus, in the series formed by introducing successive halogen atoms into β. Naphthol, the optima for disinfecting action occur at different points for different bacterial species. Tribrom β. Naphthol, with a very high disinfecting power for pyogenic cocci and the diphtheria bacillus, had little for *B. pyocyaneus* and none for the tubercle bacillus.

This same specific liability of bacterial species to the disinfecting, or growth-inhibiting, action of chemical substances is, of course, the basis of the numerous differential or enriching media, in which the selection of one species, occurring in a mixture of many, is effected by

adding some substance—e.g., a dye, or a tellurium compound—which inhibits the growth of other organisms much more effectively than that of the one which is sought. The same selective action is clearly apparent in the results published, in recent years, by Browning, Cohen, Gaunt and Gulbransen (1922), and by Fairbrother and Renshaw (1922), on the relative disinfectant action of various dyes and related compounds on different bacteria.

These selective actions *in vitro*, where there is no question of the participation of other living cells than the microörganisms under investigation, may be regarded as presenting the fundamental problem of chemotherapy in its simplest terms. Browning, Cohen and Gulbransen (1922) find, for example, that "sensitol red," a dye used in sensitising photographic plates to red rays, and having the constitution



"Sensitol red"

has a disinfectant action on *Staphylococcus aureus* more than 2,000 times as powerful as that which it exerts on *B coli*, when both are in peptone water. Their figures further indicate that the presence of serum much reduces its action on the staphylococcus, but somewhat increases its action on *B. coli*. On no system of hypothetical "tropic" properties of the dye for the coccus and the bacillus, on the one hand, and the serum proteins on the other, can these observations be intelligibly expressed. They must be accepted, for the present, simply as empirical facts. Yet the conditions are extremely simple compared with those which obtain in an experiment conducted on the living animal.

An attempt to study in partial isolation some of the factors concerned in the cure of bacterial infection by chemical agents has recently been made by Felton and Dougherty (1922 b). These authors measured, in a series of dyes, and in derivatives of the cinchona group of alkaloids, the toxicity for mice, the bactericidal action on *Staphylococcus aureus* in whole blood, and the inhibitory effect on the phagocytosis of staphylococci by leucocytes in serum. In a large series of dyes—in most of the triphenyl methane dyes tested, in safranines, phenazines and quinones, —and in that of the cinchona derivatives, they found that the inhibi-

tory action on leucocytes was exhibited in weaker dilutions than the bactericidal action. So far as these measurements can be taken to cover the factors at work in disinfection *in vivo*, they would be unfavorable to the efficacy of these substances under such conditions. A more favorable relation of bactericidal to leucotoxic properties was found in the case of some triphenylmethane dyes—p. Methoxy malachite green and ethyl violet—and in that of "proflavine" (diamino acridine), which, with the corresponding methyl ammonium derivative ("trypraflavine" or "acriflavine"), had already been found by Browning and his co-workers (1917, 1918) to have a powerful anti-bacterial action, augmented by the presence of serum, with a relatively low toxicity for the whole mammal or for its leucocytes. Another member of the same group, 2 ethoxy 6, 9 diamino acridine, was found by Morgenroth, Schnitzer and Rosenberg (1921) to give a very favorable ratio between disinfectant actions as determined, on the one hand, *in vitro*, and on the other in the tissues of a mouse, by local injection. Its hydrochloride has been introduced to commerce as "Rivanol," and has been used, by local injection, to treat cellulitis and erysipelas (Rosenstein, 1921). In spite of these favorable indications there is little, if any, evidence yet available to show that these acridine dyes can cure a bacterial septicemia. Their chief practical application has been in the local treatment of infected wounds or mucous surfaces. The action of trypaflavine on trypanosome infections will be mentioned later.

The nearest approach to the successful treatment of a bacterial septicemia by a chemical agent is found in the use, by Morgenroth (1911, 1912), of an artificial member of the cinchona series of alkaloids, ethylhydrocupreine ("optochin"), in pneumococcus septicemia. Since the alkaloid has very powerful and strongly specific inhibitory and lethal effects on pneumococci *in vitro*, its action in the infected living animal seems, at first sight, a peculiarly clear example of the action of a drug directly harmful to the parasite. Even in this case, however, there is suggestive evidence in favor of the view that the immune reaction of the host plays an important part in the cure of an infection. Thus Neufeld and Engwer (1912), Engwer (1913), and especially Moore have demonstrated the enhancement of the curative effect of a specific antipneumococcal serum by doses of optochin ineffective by themselves. The enhancement is much too great to be accounted for by a mere summation, and suggests that, short of actually killing the organisms, either the chemical or immunological antagonist may so alter them as greatly to weaken their resistance to the other.

Similarly incompatible with the conception of the action of such substances, on septicemia in animals, as a direct disinfection, was the phenomenon described by Felton and Dougherty (1922 a), working with some members of the large series of artificial derivatives of cinchona alkaloids produced by Jacobs and Heidelberger (1919 a, 1920, 1922). Felton and Dougherty found an optimum dose for the prevention of septicemia, with simultaneous injection of the drug and various multiples of the lethally infecting dose of pneumococci. If the dose of the alkaloid was increased beyond that optimum, but still well below the limit of the host's tolerance, the number of lethal infective doses which it would antagonize rapidly fell again, a number of pneumococci, which a smaller dose of the alkaloid completely suppressed, now producing a spreading infection and fatal septicemia. The authors regard this as showing that there is a reversal of relationship from bacteriotropism with the small doses to organotropism with the large. One could hardly find a better example of the desperate expedients which are necessitated by the effort to make the facts of therapeutic action fit into the rigid frame of the distribution-hypothesis. When once the coöperation of the host's defensive reaction is recognized as necessary, in accordance with the above-mentioned evidence, produced by Neufeld and Engwer and by Moore in the case of optochin, the necessity for such strained assumptions vanishes. With the lower doses, the direct antibacterial action is reinforced by the host's defensive reaction, the total effect, on the analogy of the action of optochin, being much more than a mere summation. With the higher doses, even within the tolerance of the healthy animal, the defensive reaction is impaired and suppressed, and the maximum direct antibacterial action obtainable with the alkaloid *in vivo* is inadequate, without this reinforcement, to deal with the infection.

Mention should also be made of another direction in which chemotherapeutic investigation has made contact with the treatment of bacterial infections. Chaulmoogra oil had a traditional reputation in the treatment of leprosy. The treatment was made more effective by the introduction by Heiser (1914), and improvement by Rogers (1916) of parenteral injections of the oil, or of soaps made from certain fractions (Rogers). Recently a further improvement (Macdonald and Dean, 1921) has been made by the use of artificial ethyl-esters of the separated chaulmoogric and hydnocarpic acids—peculiar fatty acids occurring in the oil, and shown by Power to be characterized by the presence of a closed carbon ring. A scientific, chemotherapeutic

basis seems to have been given to this treatment by the work of Walker and Sweeney (1920), who find that soaps of these acids, while they are harmless to the ordinary bacteria, have a pronounced antiseptic or inhibitory action on cultures of all "acid-fast" bacteria. This suggests a direct, toxic action of these fatty acids on such organisms, presumably associated with their possession of a waxy envelope. In view of somewhat similar, albeit less convincing, therapeutic claims made for the soaps of other oils, e.g., cod liver oil, which Walker and Sweeney found inert in their cultural experiments, the position cannot be said to be perfectly clear; but their results are at least highly suggestive. The difficulty of drawing any certain conclusions from a coincidence between inhibiting action *in vitro* and curative action *in vivo* is illustrated by Lewis's (1917) work on the tubercle bacillus, in which a very wide survey of dyes resulted in the discovery of several which selectively stained the bacilli, and strongly inhibited growth in culture, but produced no definite curative effects.

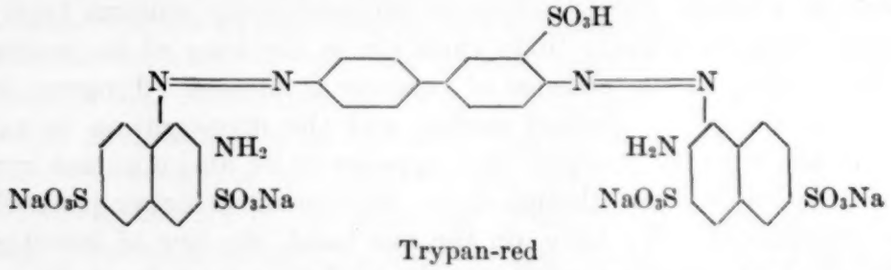
Another point of some theoretical importance, which emerges from the experiments on chemotherapy of bacterial infections, concerns the production of resistant strains. Morgenroth and Kauffmann (1912), by passage through mice treated with substerilizing doses of optochin, produced strains of pneumococci which, in the mice, were abnormally resistant to the treatment by this alkaloid. The possibility of free cultivation facilitated the study of this phenomenon of the acquisition of tolerance by bacteria under the simplest conditions. Marks (1910), by patient subculture into media containing increasing strengths of arsenious acid, produced an eight-fold rise in the resistance of a strain of hog-cholera bacillus to this substance. The treatment with arsenic raised the resistance of the organism to antimony, however, in even greater proportion—about forty-fold. The change in resistance was accompanied by modifications in the morphology and the fermentative reactions of the organism. Tugendreich and Russo (1913) similarly produced tolerance of pneumococci to optochin, and Shiga (1913) accustomed the cholera vibrio to dyes by serial cultivation *in vitro*. It is not perfectly clear whether tolerance so acquired is due to selection or to direct, heritable modification of survivors, but, in either case, the possibility of its production by the direct action of the drug, without the participation of a living host, has importance in connection with phenomena to be dealt with later.

Apart from the light which it may throw on the meaning of phenomena, which are encountered in connection with the treatment of infections

by obligatory parasites, the chemotherapy of infection by the true bacteria cannot be said to have achieved, as yet, anything of really practical importance. The use of the acridine dyes (Browning) and of the higher homologues of optochin (Morgenroth, 1917), in the local treatment of infected wounds or mucous membranes must be classed with measures of external disinfections rather than with chemotherapy in the proper sense. It is to the infections due to animal parasites that we must turn for the most characteristic and successful examples of its application, both in experiment and in practice. The spirochaets, though a strong case has been made, from the independent biological point of view, for their affinity with the vegetable bacteria, from the point of view of their infective action, and the means suited to cope with it, which is our present concern, must be ranked rather with the animal parasites.

III. Chemotherapy of trypanosome infections. A very large part of the systematic investigation of the possibility of obtaining specific chemical remedies has been directed to the cure of infections with trypanosomes. The organisms of this genus, responsible for several well-known tropical diseases of man and lower animals, are peculiarly well adapted for this type of enquiry. They are easily transmitted to suitable laboratory animals, and, when thoroughly adapted by passage, they produce an extraordinarily uniform type of infection, with remarkably little variation in the time of its progress to a fatal issue, in the absence of curative treatment. Progress has been made along two distinct routes, and the investigation in each direction has recently reached what appears to be an important summit of practical success, though there are doubtless higher peaks for future attainment. We have, on the one hand, the line of investigation of which the starting point was the trial of various dyestuffs, and of which the most recent, and apparently the most successful development, has been the introduction of the substance which, though uncolored, has important points of structural similarity with certain dyes, and which is known, as yet, only as "Bayer 205." On the other hand we have the line of investigation which started from the observation of a partial curative action produced by arsenious acid, has passed through a series of organic arsenical derivatives, and at the time appears to have reached a relative optimum in the introduction of the substance known as "tryparsamide." It will be convenient, in the first place, to sketch the general course of progress along the two routes separately.

1. The action of dyes and analogous compounds. Ehrlich's early interest in the distribution of dyes in the organs of the living animal, and his study of their specific affinities for different cell-structures, naturally led to the attempt to discover substances of this type which, by their property of becoming selectively fixed by the parasitic protoplasm, would have a parasiticidal action in doses which the host would tolerate. The first germ of the idea is seen in his observations with Guttmann, published as long ago as 1891, in which the staining of the malarial parasite by methylene blue is made the basis of an investigation of the possibility of curing a malarial infection by administering this dye. A distinct effect was obtained, but the results were not sufficiently impressive to make the dye a serious rival to quinine. Later when, with Shiga, Ehrlich began a systematic investigation of dyes as curative agents, he had at his disposal the technique of transmitting a trypanosome infection through a series of mice, as developed by Laveran and Mesnil (1902). After preliminary trials of several members of the benzopurpurin series of dyes, chosen, apparently, chiefly on account of their demonstrably long persistence in the body of a mouse into which they had been injected, the substance known as Trypan-red was obtained, through the coöperation of the dye-manufacturing firm of Cassella (Ehrlich and Shiga, 1904).

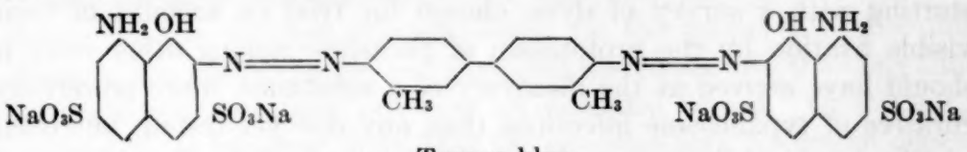


Trypan-red

This proved to have a satisfactory curative and prophylactic value for the infection of mice with the trypanosome of Mal de Caderas (*T. equinum*). It is to be noted, however, that it was relatively ineffective against the same organism when infecting the rat, and against other trypanosomes (e.g., *T. brucei*) in the mouse. It was further observed by Ehrlich and Shiga that the dye was practically innocuous to any trypanosomes, when applied to them *in vitro* even in relatively strong solutions. This chemotherapeutic paradox is one which we shall meet repeatedly. In the case of some therapeutically active substances, such as the arsenicals, we shall see that there is reasonable ground for attributing the efficacy *in vivo*, of a drug which is relatively inert *in vitro*,

to a chemical alteration into a directly active derivative taking place in the host's body. There is no ground, save that of doubtfully justifiable anology, for assuming that such a change takes place in the case of trypan-red, or, indeed, in that of any member of this series of curative dyes and analogous compounds.

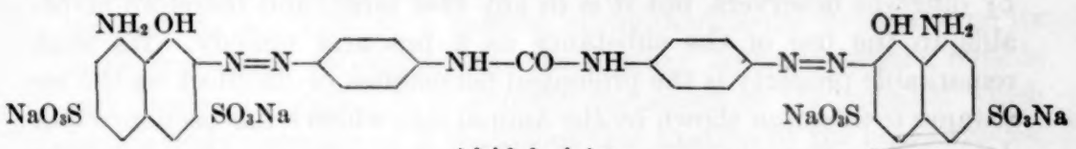
A more practically successful member of the same group was the substance introduced into chemotherapeutic investigation by Mesnil and Nicolle (1906), and subsequently known as Trypan-blue. This substance, a derivative of tetrazotized tolidine, has an obvious similarity in its general structure to trypan-red.



Trypan-blue

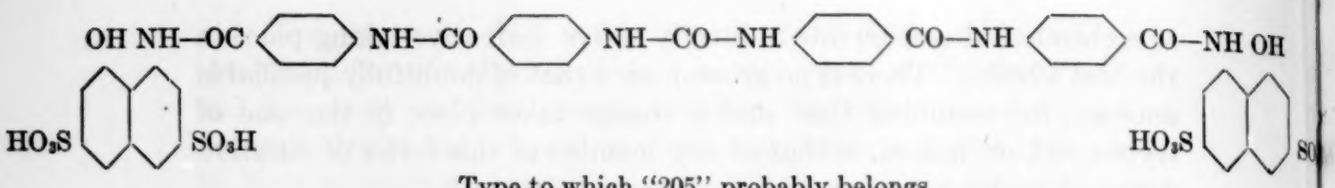
It was found to be an effective curative or prophylactic agent against *T. brucei* in mice, but its practical application has been found in dealing with another type of infection, viz., that due to intracorpuscular parasites of the genus *Piroplasma* in dogs and cattle (Nuttall and Hadwen, 1909).

Another similar substance, with which promising results were obtained by Mesnil and Nicolle, was prepared by condensing tetrazotized diamino-diphenyl urea with naphthylamin-disulphonic acid.



Afridol-violet

This dye, "afridol violet," has not itself been practically applied; its greatest interest to-day lies in the fact that it presents a point of structure, viz., the presence of an amide or urea linkage, which entitles it to be regarded as the forerunner of a series of compounds prepared in recent years by the Bayer Company, one of which, known as yet only by its serial number, "205," shows promise of much greater importance than any of the immense number of dyes which have been tested for therapeutic properties. The exact constitution of "205" is still kept secret, but it is a colorless substance, with properties which indicate a high probability, as pointed out by H. King, that it belongs to a series, patented by the Bayer Company, and having a formula of the general type:



Type to which "205" probably belongs

If this formula be compared with that of "afridol violet," it will be seen that the most important difference is the absence of the diazo-linkages by which the dye-properties are conferred. It is an interesting commentary on the validity of the theoretical conceptions with which this type of investigation may be said to have originated that, starting with a survey of dyes, chosen for trial on account of their visible fixation by the protoplasm of parasites, among other cells, it should have arrived at the discovery of a substance, more powerfully curative of typanosome infections than any dye yet tested, but itself wholly devoid of dyeing properties, and even of color. So far as they have been investigated the therapeutic properties of "205" appear to be remarkable. (Händel and Joetten (1920), Mayer and Zeiss (1920), Muhlens and Menk (1921), Wenyon (1921).) Like its colored forerunners it has no visible action on trypanosomes *in vitro*, though this absence of effect is less significant in view of the long period of about two days before its curative action begins to be manifest after its introduction into an infected animal. The ratio between the curative and tolerated dose has been assigned rather widely different values by different observers, but it is in any case large, and therefore favorable to the use of the substance as a practical remedy. Its most remarkable property is the prolonged persistence of its effect on the resistance to infection shown by the animal into which it has been injected. A single injection appears to be capable not merely of curing definitely an infection already existing in a laboratory animal, but of rendering the animal insusceptible to infection by trypanosomes for a period of weeks or even months.

Various more or less plausible hypotheses can be made to account for these anomalies in the action of "205," but there is no method yet obvious by which any one of them can be put to the test of experiment. The substance may be fixed with great tenacity by some of the tissues. The behavior of related compounds, such as trypan red and trypan blue, which are recognizable by their color, is in favor of such a supposition. The gradual and long persistent staining of the tissues of the host by these dyes is a familiar incident of their therapeutic application. (Franke (1905), Bouffard (1906).) The supposition of the mere con-

tinued presence of the substance in the body, however, is not by itself sufficient to account for the curative effect or for the artificial insusceptibility to infection, since evidence of direct lethal action on the parasites is wanting in this case, as in that of the dyes. Recourse must, therefore, be had to one or more hypotheses; but these, since they have as yet received very little support from independent evidence, cannot be regarded as much more than convenient resting-places for the mind. We may suppose, for example, that the drug only produces its effect by long-continued action, and that the relatively brief period during which its action can be observed under the microscope does not suffice for it to become visible. If that were the case an important factor in the curative efficacy would be the property of becoming fixed to the host's tissues, and liberated therefrom into the body fluids in small concentration, but over an extended period. Or it may be suggested, as has been done, that the essential curative action depends not on the direct killing of the parasites, as observed by their loss of motility and rapid degeneration under the microscope, but on destruction of their power of multiplication. This is in conformity with the observation that trypanosomes treated with a dye outside the body may lose their infectivity without visible changes in their motility or their structure. It is stated also (Busck), that trypan red will stop the multiplication of free-swimming ciliates without impairment of their other functions. We shall meet this suggestion in other connections, but the positive evidence in its favor is hardly sufficient to warrant its adoption as a general chemotherapeutic principle, or its facile invocation to explain any discrepancy between actions outside and inside the body. If it is really the secret of the paradox which immediately concerns us, it is obvious that fixation by the host's tissues, and the resulting mild, persistent action on the parasites of the gradually liberated drug, must be a factor in the effect. Another possibility is that the curative substance is not directly hurtful to the parasites, but that some parasiticidal derivative is formed from it by interaction with the host's tissues. We shall find that there is evidence in favor of such a view in the case of certain arsenical compounds, from which derivatives having a powerful, directly lethal action *in vitro* can be produced. No such evidence is available in the case of the substances which we are now considering, or indeed in that of any of the curative dyes or non-metallic compounds; the suggestion is possible, but no more. The same is true of another suggestion, namely, that the direct action on the parasites may not be due either to the substance as

administered, or to any derivative thereform, but to something produced by the tissues of the hosts in response to an action on their cells. Again, there is no evidence in favor of such a view: it is simply a convenient assumption to explain a difficulty.

It will be clear that all these possibilities, and indeed any which can be invoked to explain the curative effect in the host, of substances not obviously hurtful to the parasites outside the body, almost of necessity involve a participation of the host's organs in the curative action. Dyes were made the starting point of this line of investigation, because of the ease with which their distribution between tissues and parasites could be followed by the eye. Yet in this group the evidence so obtained is in favor of a strong affinity for the host's tissues rather than for the protoplasm of the parasites; these dyes can be seen to be strongly "organotropic," while the evidence for their directly "parasitotropic" properties is, at best, not strong.

If we take the known facts concerning the action of such a substance as "205," without reference to any particular theoretical conception, we can say that this substance, having no demonstrable direct effect on the parasites, causes their complete disappearance from the body of an animal infected with them; that for a long period after a single injection of "205" the animal is resistant to further infection by trypanosomes, and that during that period its body fluids contain something which possesses the property of curing infection; but that there is no clear evidence as to whether that something is a remnant of "205" itself, or some derivative thereof, or some antagonist produced by the host itself in response to the stimulation of its tissues by "205." The analogy of the dyes, with their demonstrable long persistence in the host's tissues, suggests, but by no means proves, that "205" is itself the substance at work.

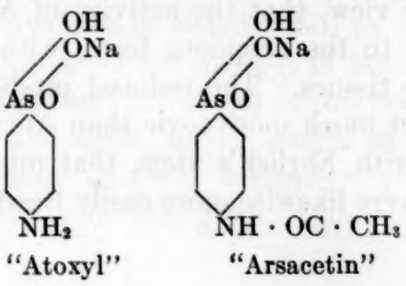
The successful experiments on the cure of infected animals by "205" have been followed by a series of apparently complete cures of well-advanced infection in man with *T. gambiense* and *rhodesiense*. A single injection has not proved adequate for the treatment of the human patient, but a course of eight or more injections has succeeded in producing what appear to be permanent cures, in a number of cases which, a short while ago, would have been regarded as quite beyond the reach of any treatment. The toxic effect of the drug itself on such patients has been a temporary and apparently not serious nephritis. (Mühlens and Menk (1921), Yorke (1921), Low and Manson-Bahr (1923).)

Another group of dyes of which the curative action in trypanosome infections has been investigated is that of the triphenyl methane dyes.

The results with these have been, on the whole, of theoretical rather than practical importance. Wendelstadt and Fellmer (1906) experimented with Malachite Green, and obtained evidence of curative action, but associated with so much local injury of the host's tissues as to make its practical application impossible. Ehrlich and his co-workers tried a number of dyes of this group and obtained promising results with Parafuchsine, though the local action on the tissues was still undesirably powerful with this substance. A chlorinated derivative of Parafuchsine, called "Tryparosan" (Roehl, 1909), was found to be more powerfully curative and at the same time much less toxic.

Another series of dyes examined by Ehrlich (1909a) and his co-workers was that containing pyronine, acridine derivatives, and related oxazines and selenazines. Of these the substance first known as "Trypaflavin" (diamino-methyl-acridinium chloride) gave curative results in mice infected with trypanosomes which, in view of its low toxicity, seemed highly promising of practical value. Its main practical use, however, has proved to be as a bacterial antiseptic, and the importance of this group of dyes, as a whole, is due more to the highly interesting results which they have given in connection with the production of tolerant strains, considered in a later section, than to their practical therapeutic value.

2. The action of compounds containing arsenic. Lingard (1899) had treated Surra in horses successfully with arsenious acid, but Laveran and Mesnil (1902), confirming Bruce's (1897) earlier observations in Africa, found that it produced only a temporary disappearance from the peripheral blood of the trypanosomes of Nagana (*T. brucei*). The application, by Thomas and Breinl (1905), of the organic arsenical derivative, which had for some time been used in treating skin diseases under the name "atoxyl," registered a definite advance. Its efficacy was soon confirmed by a number of workers, but the next step of importance was the elucidation by Ehrlich and Bertheim (1907) of the true structure of "Atoxyl" which, since it proved to be a para-amino phenyl arsenic acid, with a reactive free amino-group, formed a favorable starting point for a series of derivatives.

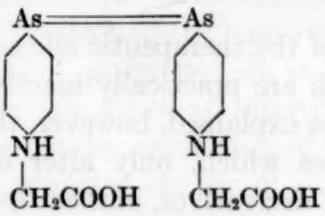


"Atoxyl" "Arsacetin"

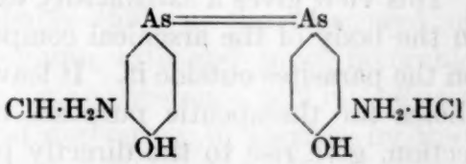
The first of these derivatives showing a more favorable experimental ratio between the tolerated dose and the dose curative of trypanosome infection, was produced by acetylating the amino-group, and named "Arsacetin." Neither Atoxyl nor Arsacetin has fully justified the early hopes of its value as a remedy in human trypanosomiasis, both having been found, in practice, to produce permanent blindness in a serious proportion of the patients treated. The study of their action, however, had results of great theoretical importance. Like the benzidin dyes, above-mentioned, these arsenical derivatives had no action on trypanosomes *in vitro* which could explain their curative action. Uhlenhuth, Hubener and Woithe (1908) reject the view that its action in the body is due to liberation of the arsenic in inorganic form—which would, indeed, be difficult to reconcile with the comparative inefficacy of the inorganic compounds when directly injected—and attribute the curative effect to the action of Atoxyl on the body cells, causing an increased formation of protective substances. The experiments of Levaditi, Brimont and Yamanouchi (1908, 1909) gave greater precision to such a conception, and suggested a participation of Atoxyl itself or some derivative of it, in the composition of the protective substance. They found that when a solution of atoxyl is mixed with an emulsion of liver, a product is obtained which is directly toxic to trypanosomes. The possibility of precipitating this toxic substance with alcohol and its instability to heat led Levaditi (1909) to conclude that it was an arsenic-protein complex, to which he gave the name "trypanotoxyl." He regarded the mechanism as a reduction of Atoxyl by the liver substance, and a combination of reduction-product with protein, the latter acting as the link for anchorage to the trypanosome. Ehrlich and Roehl (1909 b, c), however, had already shown that reduction of Atoxyl, by any ordinary reducing agent, produced, in para-amino phenyl arsenious oxide, a substance having a very intense direct toxicity for trypanosomes. According to Roehl's experiments, the function of the liver emulsion in Levaditi's was simply to reduce Atoxyl to arsenious acid, the subsequent combination of this with protein merely weakening its action. On the whole the weight of evidence seems in favor of this simpler view, that the activity of Atoxyl in the body is due to its reduction to the arsenious form, with a trivalent arsenic, by the action of the tissues. The reduced product is not only toxic for trypanosomes, but much more toxic than Atoxyl for the host; and it was in harmony with Ehrlich's view, that mice which were easily poisoned by Atoxyl were likewise more easily freed by it from trypano-

some infections than those which were more resistant to its action. Reference must be made later to some difficulties arising in connection with the tolerant strains. Meanwhile, we may note that Ehrlich's view of the action of Atoxyl departs from his original chemotherapeutic conception, in that the efficacy of Atoxyl is attributed to an immediately organotropic property. It is the reduced arsenious compound, formed in virtue of the original organotropism, which is now held to be parasitotropic.

The point of greatest importance, however, was the establishment of the superior curative activity of the compound with trivalent as compared with that containing pentavalent arsenic. This led to the production of a series of compounds containing trivalent arsenic, of which the important ones were arsenophenylglycine and dihydroxydiamino-arsenobenzene, which, in the form of its hydrochloride, has achieved world-wide celebrity under the serial number "606," and the names "Salvarsan," Arsenobenzene, Arsphenamine, etc.



Arsenophenylglycine



"Salvarsan" (606)

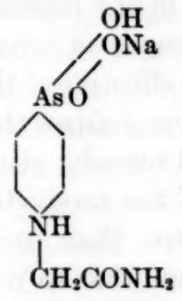
Both these substances are reduced beyond the arsenious-oxide to the arseno-condition, with a double molecule, formed by the linkage of the free valencies of the two arsenic atoms. Arsenophenylglycine gave highly promising results as a curative agent in experimental trypanosomiasis, but it failed to fulfil this promise when applied to the treatment of sleeping-sickness in man. In the case of salvarsan, which was produced by Ehrlich and Bertheim (1912), in the course of Ehrlich and Hata's (1910) search for a remedy for spirochetal infections, the powerful curative action in trypanosome infections has been somewhat overshadowed by the rapid and successful development of its use in the spirochaetal infections. Here we may note that, just as in the case of the fully oxidized derivatives of arsenic acid, we find again, in these fully reduced arseno-compounds the paradox of intense curative action on trypanosome (or spirochaet) infections *in vivo*, with no direct action *in vitro* to explain it. And again, in the case of salvarsan, we find, in the corresponding partially oxidized derivative, p-hydroxy m-amino phenyl arsenious oxide, a substance with an intense lethal action on

trypanosomes (or spirochaets) outside the body. There have again, as in the case of atoxyl, been suggestions of the formation in the body of some trypanocidal or spirocheticidal complex between the proteins of the blood or the tissue cells and salvarsan or some derivative thereof; but again the facts appear to be satisfied by the simpler theory that the parasiticidal substance is the partially oxidized arsenious-oxide derivative. This is very clearly indicated in recent papers by Voegtlin and Smith (1920) who, using a method first adopted by Morgenroth and Rosenthal (1911) for measuring the rate of disappearance of trypanosomes from the blood after curative injections, found that the effect began immediately in the case of the arsenoxid, after a definite latent period in the case of the arseno-compound, and after a longer latent period in the case of arsenic acid derivatives. These latent periods correspond with the greater liability of the arseno-compound to oxidation than of the arsenic-acid form to reduction, and the conclusion is drawn that, in either case, a preliminary to the curative effect is oxidation or reduction to the arsenoxide.

This view gives a satisfactory explanation of the therapeutic efficacy in the body of the arsenical compounds which are practically inactive on the parasites outside it. It leaves still to be explained, however, the choice for therapeutic purposes of substances which, only after injection, give rise to the directly parasiticidal derivatives, rather than these latter themselves. It is true that the arsenoxides have a much greater toxicity, not only for the parasite, but also for the host. But this alone will not explain their unsuitability for therapeutic use; for, tested on mice infected with trypanosomes, they show a high ratio between the tolerated dose and that needed to free the blood from trypanosomes, though the absolute dosage is in both cases on a lower level. The explanation must, I think, be sought by considering a factor, in the efficient treatment of infections with trypanosomes or spirochaets, which has hitherto hardly received adequate attention, namely, the factor of persistence of action. If the ready-formed arsenoxide is injected, there will momentarily be present in the blood a concentration of the direct parasiticide higher than is ever produced by its slow formation from either the more reduced or more oxidized compound; but the arsenoxide is so toxic for the host that the concentration cannot in any case be raised to a high level with safety, and the dose given at one injection must be small. To obtain the optimum effect, by administration of the arsenoxide itself, it would probably be necessary to maintain for hours or days a continuous infusion of the substance in very high dilution. This slow, persistent supply of the arsenoxide can be maintained for relatively long periods by

injecting the arsenic-acid or the arseno-derivative. In the case of salvarsan the persistence is aided by the insolubility of the free base, which must, at the reaction of the blood and tissues, be liberated and largely deposited.

This factor, of the liberation of the directly parasiticidal product of partial oxidation or reduction, is probably of importance in the action of all members of this group. And, in default of any clear knowledge of the factors which determine the rate of excretion of the parent substance, or of the rate of its change into the arsenoxide form, either of which may be affected by small changes in the nature and position of the groups attached to the benzene nucleus, there is clearly no warrant for the assumption that a change in structure, which increases the therapeutic effect, does this by increasing the preferential affinity for the protoplasm of the parasite, or by introducing an affinity for a new type of "chemoreceptor." The curative process is too complicated to be expressed in such simple terms, and involves the coöperation of the host's tissues, that is to say, some degree of "organotropic" property. It is highly probable that, in the treatment of different types of infection, the optimum rate and most favorable site for the production of the directly active substance, will in one case be provided by use of an arsenic acid derivative, in another by that of an arseno-derivative. Both have their special drawbacks. The arseno-derivatives are unstable, sensitive to exposure to air, and very difficult to obtain pure. Slight differences in the constitution, especially of the derivatives with sulphur-containing radicles attached to the amino-groups, may cause wide variations in toxicity and in therapeutic potency. Nevertheless, the arseno-derivatives are the most effective of the arsenical compounds yet obtained in the treatment of spirochaetal infections. On the other hand, the most successful arsenical compound yet tried in the treatment of human sleeping-sickness (due to *T. gambiense*) appears to be an arsenic-acid derivative, the substance known as "tryparsamide," an amide of the arsonic-acid compound corresponding to arsenophenyl glycine.



"Tryparsamide"

This substance, prepared by Jacobs and Heidelberger (1919 b, c), and selected by Brown and Pearce (1919) from a large series as the most favorable in its curative action, has now been used with highly promising results in the treatment of human sleeping-sickness. (Pearce (1921), Chesterman (1923).) It shares with "Bayer 205" the responsibility for the much more hopeful position which has recently developed with regard to the treatment of that disease. Like its parent, atoxyl, "tryparsamide" is not free from the liability to produce blindness.

3. Antimony, bismuth and other metals. Cushny appears to have made the first suggestion of a trial of compounds of antimony and bismuth, on account of their close chemical relationship to arsenic. Certain antimony compounds have been found effective in infections by trypanosomes, but not with equal certainty those of bismuth, while the reverse, as we shall see, is true of spirochetal infections. In neither case have the compounds used been aromatic complexes, with the metal attached to a benzine ring, as in the case of the arsenicals. The antimonyl—and bismuthyl—tartrates have presumably been chosen chiefly on account of their convenient solubility. Tartar emetic and its sodium analogue were used with success by Plimmer, with Thompson and Bateman (1908), and simultaneously by Mesnil and Brimont (1908 a). There are many later records of their experimental and clinical use. The action on the trypanosomes is apparently a simple and direct one, the parasites being killed outside the body by high dilutions of tartar emetic (Mesnil and Brimont). Metallic antimony suspended in oil and injected intramuscularly is apparently as effective as the antimonyl tartrate (Plimmer and Bateman), and antimony trioxide is stated to be even more efficacious. (Kolle, Hartoch, Rothermundt and Schürmann, 1913.) There appears to be no need for a more complicated assumption than that of a directly lethal action on the parasites, which must be much more sensitive to the antimony compound than are the tissues of the host. The contrast between this simple type of action and that of substances like "Bayer 205" and "tryparsamide" is the more interesting in view of the fact that, up to the time of the introduction of these latter, tartar emetic and the simple compounds of trivalent antimony, such as the trioxide, were among the more effective of the therapeutic agents tried in human trypanosomiasis. It emphasizes the possibility that the special efficacy of a complex arsenical remedy, at any rate, is due rather to the favorable rate and locality of the production from it of some simpler, directly trypanocidal substance, than to any specific affinity for the protoplasm of the trypanosome due to its molecular configuration.

The bismuth analogue has been tried in experimental trypanosomiasis with only partial (Frouin and Guillaumie, 1921) or no success (Adler, 1921). We shall see, on the other hand, that it is highly efficacious as a remedy for spirochaetal infections. Frouin and Guillaumie obtained somewhat weaker actions on trypanosome infections of mice, with cerium, yttrium and rhodium salts, but none with those of niobium.

Compounds of other metals have chiefly been used for combined treatment, together with those containing arsenic or antimony. Moore, Nierenstein and Todd (1907) advocated a combined use of atoxyl and mercury salts, but Plimmer and Thomson (1908) found that, in dosage sufficient to free the blood from trypanosomes, such treatment produced fatal lesions. Loose chemical combinations of salvarsan with other metals, such as gold or copper, have been found effective in treating experimental infections with trypanosomes, but the only compound of this kind, the "silver salvarsan" to attain practical importance has found its use in the treatment of spirochetal infections.

4. Resistant strains. One of the most interesting and theoretical important chapters in the chemotherapy of trypanosomiasis is that dealing with the development of resistant strains. When an inadequate dose of a curative agent has been given, so that a relapse of the infection occurs, further treatment is often found to be less effective, and the strain, transferred to another animal of the same species, retains its resistance and continues to do so through an indefinite number of passages. By carefully graded treatment it is possible to raise the resistance to a high level, at which it is maintained during further passages without treatment. (See Ehrlich (1907, and Browning (1907, 1908).) The phenomenon was first observed in the case of atoxyl, but was soon found to apply to other curative agents, such as the dyes. A strain resistant to trypan red was normally sensitive to atoxyl, and *vice versa*, but several such specific resistances could be developed in the same strain by the appropriate treatments.

The phenomenon at first seemed to give strong support to the conception of specific affinities for special groups in the protoplasm of the trypanosome. If the chemoreceptors for atoxyl disappeared, or were greatly reduced, the strain became proportionately resistant to the effect of this substance. When a strain resistant to atoxyl was found to be still sensitive to arsenophenylglycine, this indicated that the latter was attached to a separate "acetico-ceptor," which the treatment with atoxyl had not removed. The problem, however, has proved

to be by no means simple. In the case of an agent acting simply and directly, like tartar emetic, it can indeed be shown that the trypanosomes of a strain resistant to tartar emetic will tolerate *in vitro* a higher concentration of the drug than the original, sensitive strain (Mesnil and Brimont, 1908 b). On the other hand the resistance to tartar emetic was best produced by treatment of the infected animal with arsenious acid, while resistance to the latter itself was only obtained by very prolonged and careful treatment (Gonder, 1912). In some cases treatment with arsacetin or arsenophenylglycine has produced strains resistant not only to these substances but to tartar emetic. A still greater complication is introduced by the behavior of a resistant strain when transferred to a host of another species. Mesnil and Brimont (1908 b) produced a strain resistant to atoxyl in the mouse, and found that, when it was transferred to the rat, the sensitiveness disappeared and the strain remained normally sensitive during 40 passages through this host-species, to regain its resistance immediately when retransferred, without further treatment, to the mouse. In the dog, on the other hand, the resistance acquired in the mouse was retained. There could be no clearer evidence of the coöperation of the host's tissues in the curative action of atoxyl; on the other hand, it becomes very difficult even to speculate on the mode of their intervention. If their action were merely to reduce the atoxyl to the arsenious condition, we should have to suppose that the resistance of the parasite was to the arsenoxide; but this gives no explanation of the fact that the resistance is a property of the strain of trypanosomes and not of the hosts. Moreover there is a perplexing observation recorded by Ehrlich (1909 a) in which a strain rendered resistant, in mice, to partially oxidized arsenophenylglycine was found to be *more* sensitive to this compound *in vitro* than the normal strain. He interprets this in accordance with his view that the lethal effect visible *in vitro* is not the same as that which is effective in the body, attributing the latter to stoppage of multiplication. But such an explanation fails entirely to touch the problem presented by a resistance exhibited in one host species and not in another. Levaditi (1909), on his theory of a complex arsenic-protein poison, would regard the resistance in the mouse as specific for mouse "trypanotoxyl," leaving the strain normal in its reaction to rat "trypanotoxyl;" but again it is difficult to suppose that a specific difference, so sharp as to discriminate between mouse and rat proteins, should not exist between mouse and dog proteins. It is evident that no satisfactory solution of these difficulties is yet available, and we must be content to note the

indication which they give of the complicated nature of the chemotherapeutic process, and of the essential coöperation therein of the host's tissues.

One other highly interesting series of phenomena must receive passing mention. The resistance to drugs appeared at first to be highly specific. Later work of Ehrlich's pupils (Kudicke (1911), Gonder (1912)) showed an extraordinary reciprocal relation in the development of resistance between certain dyes, on the one hand—pyronines, acridine derivatives, oxazines, selenazines, all possessing, as pointed out by Ehrlich, an orthochinoid structure—and the arsenicals, such as atoxyl, on the other. Resistance to either of these groups seemed to confer resistance to the other; and, indeed, the most rapid and effective method of producing an atoxyl-resistant strain was found to be the injection into an infected animal of a non-sterilizing dose of a dye, such as trypaflavin. To state that the chemoreceptor for arsenic and for orthochinoid dyes must be identical, is to offer no explanation for a wholly mysterious phenomenon; it is merely to state the fact of the reciprocal action in producing resistance, in other words. Nor is it rendered, as yet, more comprehensible by the extraordinarily interesting observation of Werbitzki (1910), confirmed and extended by Gonder (1912), that trypanosomes of a strain rendered resistant to one of these dyes, though otherwise apparently normal, have lost the kinetonucleus or blepharoplast, which, according to Laveran and Roudsky (1911), can be seen to become selectively stained, and then to disintegrate under the action of the dye. Resistance to arsenicals, produced directly by ineffective treatment with these, has no effect on the morphology of the trypanosomes.

IV. Spirochaetal infections. Interest in the chemotherapy of these has, until recently, centered almost wholly round salvarsan and its soluble derivatives (neosalvarsan, sulfarsenol, sulpharsphenamine, etc.). It is beyond the purpose of this review to discuss the enormous literature on their action. We may be content to note the greater efficacy of the original "606," thrown out of true solution at the reaction of the blood, as compared with that of the derivatives soluble at neutral reaction, when both are given intravenously. It may be suggested that the factor of persistence of action is here at work, the active arsenoxide being slowly liberated over a long period from the insoluble base deposited in the tissues; and this suggestion receives support from the observation that the more soluble and readily excreted derivatives acquire an enhanced efficacy with hypodermic

injection. Some of these preparations, indeed, owe their popularity to the possibility of giving them hypodermically without causing excessive pain and induration. The increased curative action obtained by associating a silver compound with salvarsan, producing the so-called silver-salvarsan, is not yet adequately understood. It was supposed that a silver salt (nitrate or sulphate) formed an addition-compound with salvarsan, the silver and arsenic combining in virtue of residual affinities. Recent work seems to have supported the simpler, if less precise, conception, that the compound consists of silver chloride or oxide in colloidal solution, protected from aggregation by the emulsoid salvarsan. It must be left an open question whether the silver as such participates in the spirocheticidal action, or whether its association with the salvarsan favors the liberation of the directly therapeutic arsenoxide in the body.

Of fresher interest is the experimental demonstration by Levaditi and Sazerac (1921, 1922), of the curative action in syphilis, and in the closely analogous natural infection of rabbits with *Spirochaeta coniculi*, of the bismuthyl tartrate of potassium and sodium—a bismuth analogue of tartar emetic. Fournier and Guenot (1921), (1922) have used it extensively and with success in treating human syphilis. The sodium salt had already been used by Robert and Sauton (1916) for experimental treatment of spirochetosis in fowls. It will be noted that again, when arsenic is replaced by one of its chemical relatives, antimony or bismuth, the necessity for employing a complex and unstable organic derivative seems to disappear, and a relatively simple salt of convenient solubility seems to have an adequate therapeutic effect. Later experience has tended to emphasize the view that it is bismuth, administered in any form which will secure adequate absorption, which is the essential chemotherapeutic agent in this case. On the other hand, in the first observation of the action of bismuth compounds on a spirochaetal infection, in which Robert and Sauton demonstrated its curative effect in infections with *Sp. gallinarium*, these authors record that the sodium bismuthyl tartrate was without action on the parasites *in vitro*.

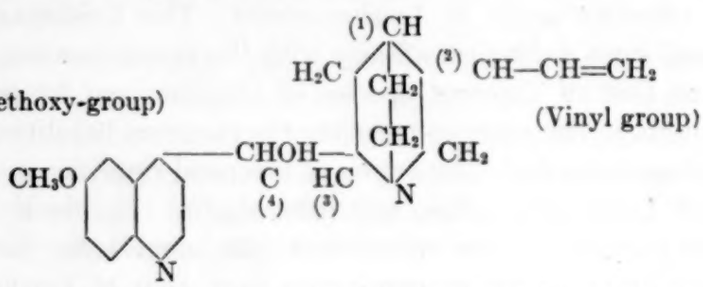
Recently Fournier, Levaditi and Schwartz (1922) have examined compounds of niobium, tantalum and vanadium. The last alone showed any effect on experimental or clinical syphilis, but these effects of vanadium were comparable to those obtained with the analogous bismuth salts. Pröscher, Seil and Stillians had already (1917) shown the effect of vanadium on syphilis.

We may note that, in their long series of experiments, which led ultimately to salvarsan, Ehrlich and Hata tried a number of dyes in spirochetal infections, without any results of value. "Bayer 205" which, though not a dye itself, may be regarded as the most successful achievement, up to the present, of chemotherapeutic investigation on compounds of this type, appears to be without effect on spirochetal infections. Up to a certain point, and especially in connection with the arsenical remedies, trypanosomes and spirochaets appear to show a certain community of response to therapeutic agents; thereafter divergence is apparent, the lines of investigation, which at the moment show the greatest promise for new development, being, in the one case, in the direction of complex organic substances related to the dyes, in the other case, in the direction of relatively simple salts of the metals related to arsenic and antimony.

V. Leishmaniasis. The successful treatment of Leishmania infections has hitherto been practically limited to the intravenous injection of tartar emetic and its sodium analogue. The use of these salts appears to have resulted from empirical trial by different workers (Vianna (1913), Carini (1914), Cristina and Caronia (1915), Rogers (1915)), working merely on the broad analogy of its previous use in other protozoal infections. It was remarkably successful, though the arsenical remedies had been relatively valueless. At the time of writing the first news is published of the successful treatment with "Bayer 205" of a case of kala-azar, which had resisted treatment by tartar emetic (Yorke, 1923). Simultaneously there is published by Lindenberg (1923) an account of the treatment by "Bayer 205" of the cutaneous Leishmaniasis of Brazil. Cases can apparently be cured by hypodermic injection, but local application of "205" to the sore is without effect: a most significant observation, if confirmed. This is but one example, out of a number, which will present themselves, of the lack of correspondence between the facts of chemotherapy and the accepted zoölogical affinities. "Bayer 205" is highly effective in certain trypanosome infections, quite ineffective in others, but apparently effective again in Leishmaniasis. The Leishmania parasite has, indeed, some zoölogical affinity with the trypanosomes, but hardly so close as that of different species of trypanosome for one another. We may note, in the same connection, the common liability of trypanosomes and spirochaets to the action of arsenical remedies such as salvarsan, which Leishmania does not fully share. No theory as to the systematic position of the spirochaets can suggest for them a closer biological affinity to the trypanosomes than that of Leishmania.

VI. Malaria. For the treatment of malaria the modern development of chemotherapy has effected practically nothing. Since cinchona bark was brought to Europe in the 17th century, real improvements in the treatment of malaria have been almost limited to improvements in the administration of this drug and its alkaloids, guided in the more recent period by the recognition of the malarial parasites and more accurate knowledge of the chemistry of the cinchona alkaloids. Ehrlich and Guttmann's (1891) trial and recommendation of methylene blue has been mentioned. Later observations have not supported its claim to rivalry with quinine, nor have any of the newer remedies for other protozoal infections, such as salvarsan, acquired an importance in the treatment of malaria at all comparable with that of quinine. The special reputation of quinine, as compared with the other natually occurring cinchona alkaloids would appear to be largely artificial. It apparently arose at a time when quinine and cinchonine were the well-known and characterized alkaloids, the latter contrasting unfavorably with quinine on account of its toxicity for man. In recent years, with improving knowledge of the cinchona alkaloids, others have been tried, with results which seem to indicate that some of them —hydroquinine, quinidine, cinchonidine—are at least as effective in the treatment of malaria, and not noticeably more toxic. (McGilchrist (1915), Giemsa and Werner (1914), Acton (1920).)

The point has practical importance since the whole policy of the artificial cultivation of cinchona has hitherto been governed, both in selection of soil and climate, and the selective breeding of the trees, by the supposed necessity of securing a maximum yield of quinine in proportion to the other alkaloids. Theoretically it is interesting as showing that neither the vinyl group, which is reduced to an ethyl group in hydroquinine, not the methoxy-group, which is absent in cinchonidine, nor the precise stereometric configuration of the quinine molecule, since quinidine differs in its arrangement in relation to at least one of the four asymmetric carbon atoms, is essential to the therapeutic action of quinine in malaria.



Quinine. (1), (2), (3) and (4) are the 4 asymmetric carbon atoms.

With the exception of hydroquinine, none of the many artificial derivatives which have now been made from these cinchona alkaloids have proved to be even as good as the natural ones in treating malaria: they have found their practical uses in other directions, in in the treatment of bacterial infections. The main question of chemotherapeutic interest, therefore, is still that of the manner in which quinine and its similarly acting relatives produce their antimalarial effect. It is a curious item in the history of the subject that Binz, as long ago as 1867, should have correctly predicted that the cause of malaria would prove to be a protozoön, on the quite inadequate ground of his observation that quinine, with its traditional value in malaria, had a lethal action on free-living protozoa (paramecium etc.) in relatively high dilutions. It would have been as logical, from the failure of quinine to cure tropical dysentery or sleeping sickness, to conclude that the infective agents in these diseases were probably *not* protozoa. After the discovery of the malarial plasmodia various attempts were made to observe the effect of quinine on the parasites. Many such observations were directed to following the degenerative changes in the parasites during the treatment of the patient with quinine; these, of course, leave quite open the mode of action of the remedy, whether directly on the parasite, or indirectly, by stimulating the defensive response to the host. Observations on the effect of quinine added to malarial blood *in vitro* have not produced any conclusive evidence in in favor of a direct parasiticidal action of the alkaloid. (Laveran (1891), Dock (1891), Marchiafava and Celli (1886), LoMonaco and Panichi (1900).) The last named authors appeared to regard the effect of quinine, in medicinal doses, as a stimulation of the young parasites, leading them to leave the red corpuscles and so to become exposed to the unfavorable conditions of the blood-plasma. This observation has an obvious relation to the interesting theory of quinine action much more recently put forward by Morgenroth (1918). This observer, as the result of tests made by physiological and bacteriological methods, came to the conclusion that, when quinine is added to shed blood, a very high percentage of it becomes extracted by the corpuscles, the serum or plasma being left almost free from quinine. On the basis of these observations Morgenroth puts forward the suggestion that quinine owes its curative action in malaria to its *organotropic* properties, especially its affinity for the red corpuscles. He leaves it an open question whether the quinine, so located, acts in relatively concentrated form on the already intracorpuscular parasites, or whether, as

he thinks more probable, the quinine, being concentrated on, or in, the surface layer of the corpuscle, repels the merozoites resulting from the asexual reproductive cycle, so that, being denied access to the place of their further growth, they perish in the plasma. While it is of interest to find Ehrlich's former colleague thus frankly adopting a view, which attributes the curative action of quinine to an organotropic and not a parasitotropic property, it must be borne in mind that none of the numerous determinations, of the distribution of quinine between corpuscles and plasma, which have been made by chemical means, give any support to Morgenroth's deduction from his biological tests. Ramsden, Lipkin and Whitley (1918) found a ration of 2 or 3 to 1 in favor of the concentration in the plasma, while later determinations, by Acton and King (1921), show a practically equal distribution throughout the blood mass. The discrepancy is obviously one which demands further investigation. For our present purpose it is sufficient to note that no simple conception, of directly lethal action on the malarial parasites, will fit the knowledge yet available as to the nature of the curative action of quinine in malaria.

VII. Amoebiasis. As in the case of malaria, the only drug which, even to-day, can be stated to have an indisputable and specific action on tropical dysentery, was introduced to Europe from South America in the 17th century for the treatment of that disease, more than two centuries before the organism responsible for it was discovered. The alkaloids of ipecacuanha root like those of Cinchona bark, have now been isolated and characterized; and again we find among them, in emetine and cephaeline, principles having an antiamebic action more potent and specific than that of any other known substance, including such artificial derivatives of emetine as have hitherto been prepared. The *Entamoeba histolytica* is an organism much more readily accessible for direct observation of effects *in vitro* than the malaria parasite; and until a few years ago the evidence seemed to point distinctly to the simplest possible mechanism for the curative action of emetine in amebic infections, namely, a directly lethal action on the amebae. Vedder (1911) showed that the alkaloids, in relatively high concentration (1 in 100,000) would kill free-living amebae, though Lyons (1912), who made similar experiments, was doubtful as to the adequacy of the results to explain the therapeutic action. Rogers' (1912) experiment, which indicated that *Entamoeba histolytica*, fresh from the human intestine, was killed in a few minutes by emetine in a dilution of 1 in 100,000, suggested that there was no need to look further than simple,

direct action on the parasite for an explanation of the therapeutic action in the patient.

Repetition of such observations, and their extension to the other alkaloids of ipecacuanha, to artificial derivatives therefrom, and to other substances, have made this simple view quite untenable. Kuenen and Swellengrebel (1913) observed a lethal action of emetine on *E. histolytica*, but it needed from 1 in 10,000 to 1 in 5,000 to produce the effect, and periods up to many hours for its development. The experiments of Kolmer and Smith (1916), and of Walters, Baker and Koch (1917) also showed a much lower order of activity for emetine on parasitic or free-living amebae. Dale and Dobell (1917) studied the effects of a number of these alkaloids on the fresh entamebae in the test-tube, obtaining the material mostly from cats infected from human cases of dysentery. Some of the same substances have been clinically tested on man by G. C. Low. A very curious contrast is revealed. While Dale and Dobell did not find emetine without effect on the isolated amebae, both it and cephaeline were far less active than Rogers' statement had suggested, and less so than other alkaloids, such as quinine and harmaline, which have no curative action in an amoebic infection. This has, in essence, been recently confirmed by Allen (1920), using amebae direct from human patients. On the other hand, one of the minor natural alkaloids from ipecacuanha, methylpsychotrine, and several artificial derivatives, such as demethoxyemetine, were found by Dale and Dobell to exhibit a more powerful toxic action for the isolated amebae and a relatively very low toxicity for the mammal. These, when tested on human sufferers from dysentery, all failed entirely to justify this promise of therapeutic value; so far as results at present available warrant a conclusion, it would be that the therapeutic efficacy of these alkaloids, in dysentery, has no relation to their toxicity for *Entamoeba histolytica* outside the body, but is, roughly, in direct proportion to their toxicities in man.

Another curious fact which emerged was that a strain of Entamoeba which was readily susceptible to treatment by emetine in the human patient, was completely resistant to emetine in kittens, to which it had been transferred from man before the treatment was applied. The close connection of the curative action of emetine with the behavior of the organism as a tissue-parasite is made clear by the fact that treatment with emetine eliminates infection with *E. histolytica* and leaves undisturbed the saprophytic entamebae and allied forms (*Entameba coli*, *Endolimax nana*) living in the contents of the colon. We are,

once more, almost compelled to suppose that the tissues of the host play an essential part in the curative action which the drug initiates. It is, of course, just conceivable that emetine, while not visibly affecting the motility and vitality of the amebae, when applied in high dilutions, has nevertheless a powerful inhibiting action on their reproductive activity, which, since artificial culture has not proved possible, cannot be detected outside the body. So far as experiments on free-living amebae in culture can provide sound evidence on this point, it is not in favor of such a view. Pyman and Wenyon (1917) found, indeed, an inhibiting action produced by emetine and cephaeline and none by psychotrine, which has no curative action. They observed, however, equally good inhibition of multiplication by N. methylemetine, the curative effect of which is, at best, very slight. It would be rather a forced assumption, moreover, to suppose that alkaloids nearly related to emetine, and having a more pronounced immediate and visible action, should be devoid of the inhibiting action on reproduction; and the assumption could not, in any case, explain why emetine inhibits the reproduction of the entameba in man, but not that of the same strain in the cat. To suggest, as an explanation, coöperation of the host's tissues, in a manner concerning which the knowledge yet available affords not even a hint, is unsatisfactory; but it seems at present the only alternative to a completely agnostic attitude.

VIII. Helminthiasis. Strictly speaking we ought to include under the heading of "chemotherapy" the removal of parasitic worms from the alimentary canal, by administering, by the mouth, substances which kill or paralyze the worm, without producing serious symptoms of poisoning in the host. The conditions, however, are so different from those which govern the treatment of infections by parasites of the blood and tissues, and the action involved is so obviously and beyond question a direct poisoning of the parasite, that the inclusion of this type of treatment in this review would be misleading rather than illuminating. The case is different with infections by worms of the blood and tissues: here the problems of treatment closely resemble those encountered in dealing with protistal infections. Such success as has been attained has resulted from the empirical trial of remedies which have been found effective in infections with protozoa or spirochaets. Infection with the guinea-worm can, apparently, be cured by intravenous administration of salvarsan or tartar emetic. (Jeanselme (1919), Montpellier and Ardoin (1919), Macfie (1920).) But the most striking and best authenticated success has been attained in treating

infections with Bilharzia (Schistosoma) by tartar emetic, injected intravenously. This was tried by Christopherson (1918), (1919), (1920) in 1918, who was induced to make the attempt by his observation of its success in Leishmaniasis. Before this successful trial, a chemotherapeutic attack on Bilharzia infections had been regarded as an almost hopeless enterprise, since the symptoms constituting the essential disease are produced, not by the adult worms in the circulation, but by the innumerable, irritating and highly resistant ova, finding their way to the exterior through the tissues of the bladder and the rectum. It is still a matter for surprise that the eggs should be killed by the antimony treatment, and that, with their death, the irritating action should cease. The evidence as to the mode of action is still incomplete. Christopherson describes experiments, which he regards as proving a direct lethal action of the tartar emetic on the ova and the free-swimming embryos (miracidia). It cannot be said that, having regard to the concentrations employed, they carry a strong conviction of the correctness of his view.

This theory of directly lethal action is rendered more difficult by the fact that a number of observers (Diamantis (1917), Mayer (1918), Bonne (1919), Debbas (1920), Erian (1919), Tsykalas (1921)) have made very similar claims for emetine, administered hypodermically or intravenously, as a cure for bilharziasis of the bladder or the rectum. On the other hand, it is at least extremely doubtful (Low and Gregg, Macfie) whether tartar emetic has, on Filaria infections, the curative effect which Rogers had suggested.

CONCLUSION

In the foregoing pages an attempt has been made at an impartial summary of the facts which must be covered by any general theory of the mode of action of specific chemical remedies for infections. Already in the early days of this type of investigation, when attention was focussed on the effects of certain chemicals on infection with trypanosomes, facts were constantly presenting themselves which could only with difficulty, and with aid of subsidiary hypotheses, be reconciled with the idea that the curative substance acted in virtue of its differential affinity for the parasitic protoplasm. The contrasts between effects *in vitro* and those *in vivo;* the phenomena of acquired resistance, specific for the infected host as well as for the infecting strain of parasites, and showing, at the same time, discrimination between nearly related remedies and community as regards others having no chemical

similarity; these phenomena were, and still are, difficult to bring within the scope of any simple type of explanation. The difficulty has greatly increased during recent years, as the methods of chemotherapy have spread to the treatment of other types of infection. When we are dealing with organisms of one genus, or of a few closely allied genera, the postulation of different chemoreceptors, corresponding to the different kinds of compound found therapeutically effective, though it does not convey more real information than the record of the results observed, does not involve any apparent absurdity. When, however, we try to apply the terminology to the more recent extensions of chemotherapy, and find it necessary to suppose, for example, that the *Bilharzia* worm has antimony-receptors in common with trypanosomes or *Leishmania*, and emetine-receptors in common with the dysentery ameba, it becomes increasingly difficult to use the nomenclature with complete solemnity. Ehrlich's theory will always deserve the credit of having provided a vigorous stimulus to the investigation of problems which, without some kind of working hypothesis, might well have seemed beyond the reach of an experimental attack. That being admitted, it is necessary, on the other hand, to admit that few of the successful results hitherto obtained have been obtained by a consistent application of the theory. Some of them seem, indeed, to be the result of experiments which a serious acceptance of the theory would have discouraged. As new successful applications have become more frequent, their basis has become increasingly empirical. It is difficult to resist the conclusion that a new theoretical foundation is required for further orderly building, and that this will have to take fuller account of the great complexity of the therapeutic process, and especially of the coöperation therein of the infected host. And, if this should mean some measure of reunion between "chemotherapy" and the parent pharmacology, from whose rather unenterprising tradition it claimed to be free, the result can only be to the advantage of therapeutic science.

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INTERMEDIARY METABOLISM OF CARBOHYDRATES

PHILIP A. SHAFFER

The Laboratory of Biological Chemistry, Washington University Medical School, St. Louis, Mo.

Much the greater part of the energy made use of by man and other animals to warm their bodies and to do muscular work is obtained by the oxidation of glucose and other simple sugars, derived from the digestion of various carbohydrates contained in the food. In ordinary diets of human beings from one-half to two-thirds or even more of the oxidizable foodstuffs are sugars and starches, which after digestion are absorbed from the intestine chiefly in the form of glucose. Fructose and galactose are also absorbed and utilized, though in much smaller quantities.

Besides the food carbohydrate, about 60 per cent of the protein amino acids and about 10 per cent of fat (glycerol) (129) are believed to be converted into glucose in the normal course of their metabolism. Fatty acids also, according to the view of some investigators, are convertible into glucose (74), though the burden of evidence appears to be contrary to this conclusion. The metabolism of glucose thus represents, from a quantitative standpoint, much the most important chemical reaction taking place in the body, and for the purposes of this brief survey, its transformations will be regarded as representing the intermediary metabolism of carbohydrate.

In addition to starch and common sugars which yield on hydrolysis or digestion the three hexoses named above, plant tissues used for food by herbivorus animals contain large amounts of polysaccharides which on hydrolysis by acid yield sugars of five carbon atoms, the pentoses. These pentosans undoubtedly furnish a very important source of energy to grazing animals, but it is probable that their digestion is largely in the nature of fermentation by flora of the intestinal tract, with the formation of acids and other products rather than the constituent pentose sugars. Insofar as this is the case the carbohydrate characteristics are lost before the products enter the blood stream. In these animals glucose, glycogen and lactose may be formed by synthesis from the fermentation-digestion products of pentosans, which thus by a

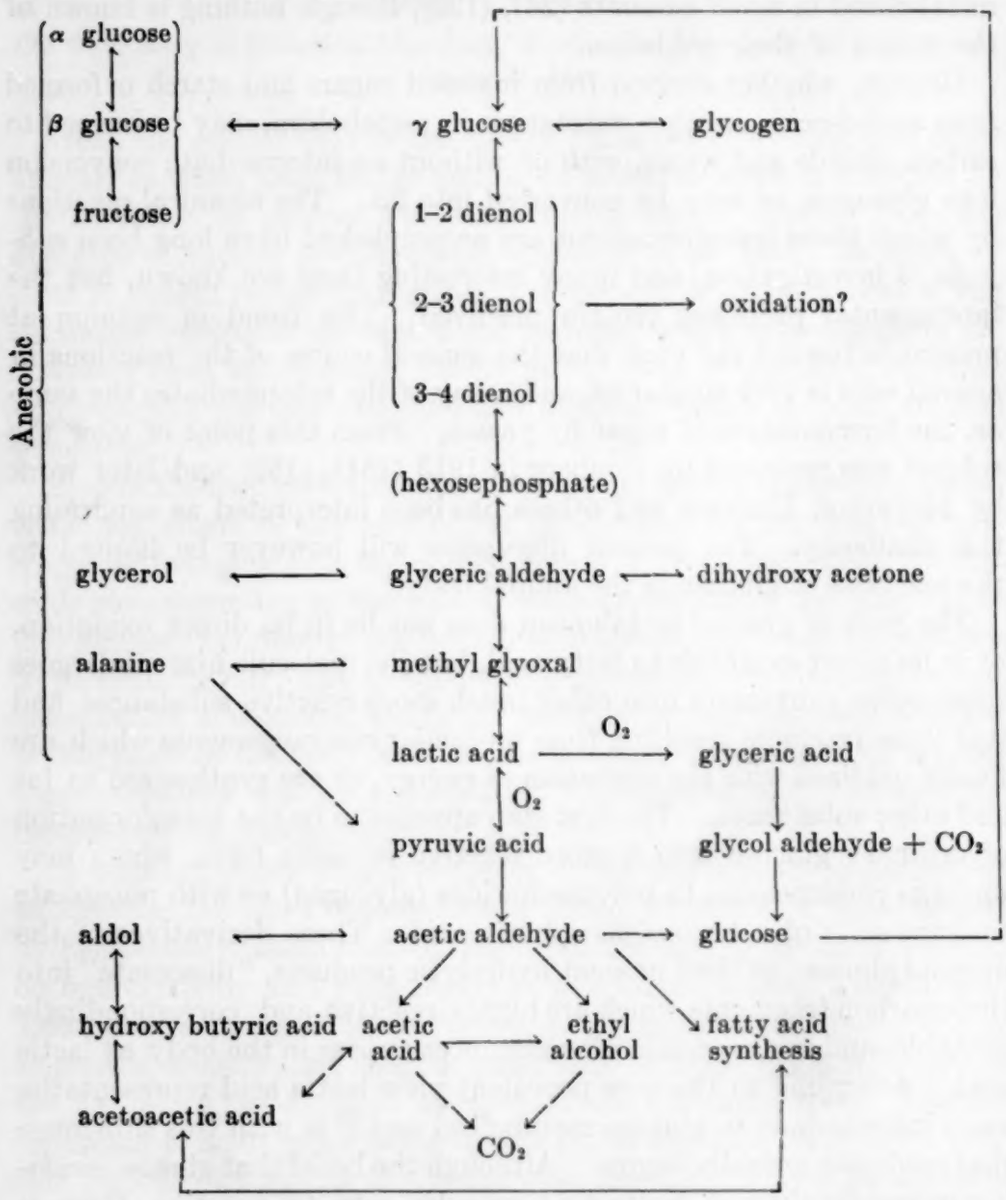
round-about transformation play an important rôle in the carbohydrate metabolism. Pentosans are scarcely if at all digested or absorbed by man, and therefore have little part in human metabolism. When the pure pentoses are fed a part has been found to be excreted unchanged; neither arabinose or zylose are converted into glucose in the body (13). It appears that these sugars and the methyl pentose, rhamnose may be metabolized in small amounts (24), (122), though nothing is known of the stages of their oxidation.

Glucose, whether derived from ingested sugars and starch or formed from amino-acids or other substances in metabolism, may be burned to carbon dioxide and water, with or without an intermediate conversion into glycogen, or may be converted into fat. The chemical reactions by which these transformations are accomplished have long been subjects of investigation, and many interesting facts are known, but the fundamental problems remain unsolved. The trend of opinion at present is toward the view that the general course of the reactions in animal cells is very similar to, and many of the intermediates the same as, the fermentation of sugar by yeasts. From this point of view the subject was reviewed by Neuberg in 1913 (151), (152) and later work by Meyerhof, Embden and others has been interpreted as confirming this similarity. The present discussion will however be limited to the reactions of glucose in the animal body.

The path of glucose metabolism does not lie in its direct oxidation, or in its direct synthesis to fatty acid, but the molecule first undergoes progressive conversion into other much more reactive substances and it is these products resulting from molecular rearrangements which are finally oxidized with the liberation of energy, or are synthesized to fat and other substances. The first step appears to be the transformation of ordinary glucose into a more reactive isomeric form, which may undergo condensation to polysaccharides (glycogen) or with phosphate to form salts of a hexose phosphoric acid. These derivatives of the original glucose, or their nascent hydrolytic products, "dissociate" into three carbon fragments which are highly reactive and correspondingly unstable, and under certain circumstances appear in the body as lactic acid. According to the now prevalent view lactic acid represents the main intermediate in glucose metabolism and it is with this substance that oxidation actually begins. Although the belief that glucose *oxidation* proceeds through lactic acid may be open to question, there is conclusive proof that the conversion, glucose $\rightleftarrows$ lactic acid, does normally take place in both directions in the body, and that the reactions

concerned in this transformation are fundamentally related to carbohydrate metabolism.

As a basis for the following discussion the reader is asked to consult the following diagram. Some of the reactions indicated are more or less hypothetical and the diagram is intended only as an aid in orienting ourselves in the maze of evidence which requires consideration.



It will be noted that the first group of reactions at the top of the diagram involve only reversible rearrangements within the glucose molecule and its condensation to polysaccharides (glycogen), in which little

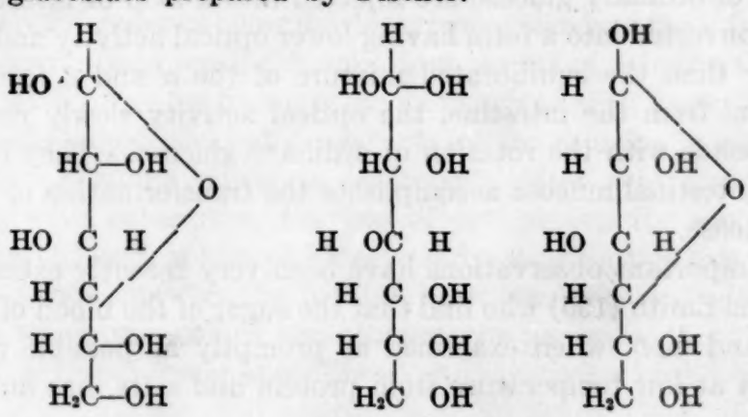
or no energy change takes place. Recent evidence indicates that these transformations although not extensive as to molecular structure may nevertheless be of fundamental importance.

The reactions of the second group from glycogen or γ glucose to lactic acid are probably also reversible and involve no oxidation and but little energy change, but do involve a splitting of the six carbon molecules into three carbon fragments. The loss of energy in the sum of the reactions from glucose to lactic acid (including neutralization with bicarbonate or phosphate) is at most about 190 calories per gram of lactic acid (141) which is only about one-twentieth of the calorie value of the glucose from which it was derived.

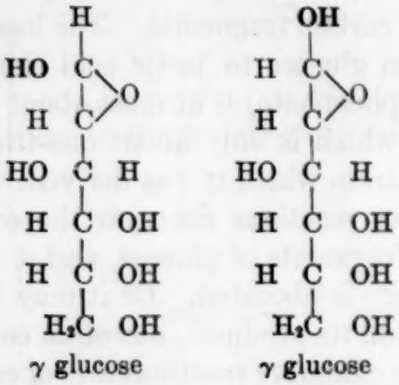
The third group of reactions concerns the oxidation of the three carbon dissociation fragments of glucose, and it is in these stages that the bulk of the energy is liberated. Or it may be that the oxidation is not of lactic acid and its products, but of an earlier derivative of the parent glucose. The oxidative reactions are, of course, exothermic and are, in the main, irreversible in the body.

We may first consider the evidence as to different forms of glucose.

THE DIFFERENT FORMS OF GLUCOSE. It has long been known that the optical activity of freshly prepared solutions of ordinary glucose gradually becomes less, and only after some hours attains a value corresponding to the specific rotation of $+52.5°$. This phenomenon (mutarotation) which is exhibited also by other reducing sugars, studied especially by Lowry (127) and by Hudson (96), is due to the slow conversion of α-glucose, a form predominating in ordinary solid preparations of the sugar, into β-glucose. The final rotation is the result of an equilibrated mixture of the two forms, which were isolated by Tanret (190). The formulae generally assigned to these forms are given below and represent butylene oxides corresponding to the α- and β-methyl glucosides prepared by Fischer.



The conversion of one form into the other is believed to take place through the intermediate formation of the aldehyde structure by the breaking of the butylene ring and its reformation. Although slow at low temperatures and in neutral solutions, the conversion is rapid on warming and is practically instantaneous in the presence of even very slight alkalinity.



γ glucose γ glucose

Besides these ordinary forms there is good evidence that glucose exists also in much more reactive modifications. Fischer (59) and Irvine (98) have identified two additional methyl glucosides as the derivatives of an ethylene oxide structure of glucose, designated γ glucose. The parent glucose according to Irvine, Fyffe and Hogg (98), (99), and Armstrong and Hilditch (5), (6), readily undergoes condensation, is rapidly oxidized by permanganate, and is much more reactive than ordinary glucose. Although γ glucose has not been isolated, its existence and its greater reactivity appear to be established. These more reactive forms have acquired very great biochemical significance through several recent discoveries which suggest that the α and β butylene-oxide forms must be converted into the γ forms before glucose is utilized in the body. Hewitt and Pryde (85) have found that when solutions of ordinary glucose are injected into a loop of intestine it is rapidly converted into a form having lower optical activity and greater reactivity than the equilibrated mixture of the α and β forms. On withdrawal from the intestine, the optical activity slowly rises until it corresponds with the rotation of ordinary glucose. They conclude that the intestinal mucosa accomplishes the transformation of α and β into γ glucose.

These important observations have been very recently extended by Winter and Smith (195) who find that the sugar of the blood of normal animals and men, when examined as promptly as possible after its separation at low temperature from protein and salts, has an optical

activity much below that which corresponds to ordinary equilibrated glucose. On standing the optical value rises until after a day or two it becomes constant and agrees with the value expected from the determination of glucose by copper reduction methods. They were unable to detect fructose in the separated blood sugar. The phenyl hydrazine ozasone had the melting point and the crystal form of glucosazone.

Furthermore, blood sugar during alimentary hyperglycemia following the ingestion of 150 grams of fructose and after 100 grams of glucose, showed the same discrepancy between optical rotation and copper reduction values; the copper values remained constant, while the optical values increased until the two agreed. Presumably, therefore, both glucose and fructose are transformed during absorption or soon thereafter into the same form. The sugar obtained from the blood of human diabetics on the other hand did *not* show the low optical rotation, but agreed with the copper reduction values. In one case the initial rotation of the diabetic sugar was *higher* and gradually decreased to the copper value. But after the injection of "insulin" (9), causing a decrease of hyperglycemia and of glycosuria, the sugar extracted from diabetic blood was found to be *similar to that of normal blood,* the initial rotation being lower than corresponds with the copper reduction, and increasing on standing (34). The authors interpreted their observations as indicating the conversion in the body of the normal subject of α-and β-glucose into the more reactive γ-form having a lower specific rotation,—a conversion which is not accomplished by the diabetic organism. The same workers have expressed the hypothesis that this conversion is essential for the oxidation of glucose, and that the inability to effect this transformation is the basic defect in diabetes. They report that preparations of pancreas ("insulin"), when added to liver extracts bring about a decrease in the optical activity of glucose which they interpret as being due to its conversion into the γ-form (196).

The observations recall the important results of Admont Clark (17) who found that on perfusion of dog's pancreas with Locke's solution containing glucose, the optical activity of the solution is reduced but its copper reducing power is unaltered. After acid hydrolysis, or standing with antiseptics, the loss of optical activity was regained. Perfusion of heart, spleen or kidneys did not cause this loss of optical power. When however the beating heart was perfused with Locke's solution which was passed also through the pancreas, there was a decrease in reducing power as well as of optical activity, both being in part

regained on acid hydrolysis. Besides a consumption of glucose, a part was converted into a substance having lower optical activity than ordinary glucose. The osazones prepared from pancreas perfusates had melting points distinctly different from that of glucosazone, being from 200.5°C. to 203°C., while after acid hydrolysis of the same perfusates, the osazones then obtained melted from 203.5° to 206.5°. Clarke concluded that the pancreas furnished to the perfusing fluid an enzyme which changes glucose into a "simple form of polysaccharide" with lower optical activity but of the same reducing power as glucose. The heart was able to utilize some of the sugar after it had been transformed by the pancreas enzyme, and a part was further condensed to polysaccharides, the optical activity and reducing power of which were increased by acid hydrolysis.

In addition to demonstrating a reversible decrease in the optical activity of glucose on perfusion through pancreas, which, in the view of Hewitt and Pryde and of Winter and Smith, may be explained by its transformation into γ-glucose, Clark's results also point clearly to the necessity of this transformation before glucose can be oxidized by the heart muscle. Recent experiments by Hepburn and Latchford (84) demonstrate an increased rate of glucose consumption by the beating heart when insulin is added to the perfusion fluid.

Referring to our diagram it will be noted that the investigations cited above have to do especially with the transformation of α- and β-glucose into another form, possibly γ-glucose. According to Irvine, one of the characteristics of γ-glucose is its tendency to undergo condensation. This suggests that it may be the γ-glucose, formed by the intervention of pancreas activator, which forms glycogen. Banting, Best, Collip, Macleod and Noble (9) find that the amount of liver glycogen in depancreatized dogs is very markedly increased by insulin injections, whereas , as is well known, the amount of glycogen in diabetic livers is ordinarily small. Macleod (132) takes this to mean that glycogen is an essential preliminary stage in sugar utilization, and that glycogen is not formed in the absence of the pancreatic hormone. This is in line with the results of Barrenscheen (10), who found that on perfusion of the livers of depancreatized dogs, added glucose and fructose do not form glycogen, while this formation does take place on similar perfusion of livers of normal animals.

From the evidence cited above the hypothesis appears attractive, that α- and β-glucose is converted under the joint influence of tissues and of the pancreas hormone into γ-glucose, which may then be poly-

merized to glycogen or transformed into other derivatives capable of oxidation, both of which fates are closed to the molecular configuration of ordinary glucose. According to this view the effect of the pancreas hormone is located with the early molecular rearrangements within the glucose molecule, perhaps in initiating its enolization, and especially the formation of 1–2 dienol, which may be regarded as similar to γ-glucose.

Brief mention may be made of another sort of condensation which may or may not have to do with glycogen formation. It will be recalled that Hirsch (91), Arnheim and Rosenbaum (7) and especially Cohnheim (23) observed a disappearance of glucose by the joint action of muscle juice and pancreas extracts. The observation was confirmed by Hall (80), Dewitt (35) and Levene and Meyer (114). The last named investigators, however, showed that the loss of copper-reducing power was the result not of "glycolysis" but of a condensation. After the action of muscle + pancreas extract upon strong glucose solutions, they were able to isolate an osazone, which on analysis appeared to be that of a disaccharide. After the digestion had taken place with a lowering of reducing power, hydrolysis of the resulting sugar solution by acid, or hydrolysis by the tissue enzymes after dilution, increased the copper reduction values to that of the controls before digestion. Similar condensation was observed of fructose, but not with mannose, xylose, ribose and lactose. The condensation of glucose was not found to take place by muscle or other tissues alone, but required the addition of pancreas extract (or, in case of the dog, spleen extract, (117)).

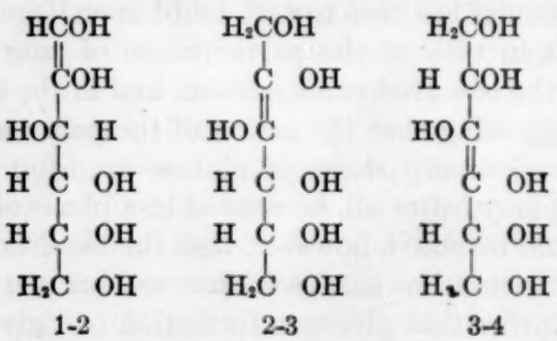
This phenomenon appears not to have been further investigated, and its significance is at present not clear. The effect of Levene and Meyer's (117) results has been to cast doubt upon its relation to glucose catabolism, but in view of the participation of pancreas extracts in accomplishing the observed condensation, and in the light of the more recent work suggesting that the action of the pancreas hormone may be upon the *preliminary stages* of glucose transformation, it seems possible that it may, after all, be related to a phase of glucose metabolism. It should be noted, however, that the conditions under which this type of condensation has been observed are very different from those under which either glycogen formation or "glycolysis" by conversion to lactic acid have since been found to take place. (Antiseptics, long incubation and high sugar concentration.) Sterile suspensions of leucocytes or of kidney tissue in phosphate solutions convert added glucose, fructose, or mannose into d-lactic acid (115, 116, 117), but the presence of toluol wholly prevents this action.

The action of alkalis on glucose. Although relatively stable in acid or neutral solutions, under which circumstances glucose requires strong reagents for its oxidation, it becomes at once highly reactive in the presence of alkalis and will absorb atmospheric oxygen (135). Even if oxygen be excluded the optical activity declines (142) and the sugar is converted into a large number of different substances. The reactions which take place in alkaline solutions have been found to be of great complexity. Some of the points resulting from these extensive investigations may be stated as follows.

In exceedingly dilute alkali there occurs a very rapid transformation of freshly dissolved glucose or other sugars, with a change in optical rotation of the solution, the change being due merely to the partial conversion of α-glucose into the isomeric β-form (mutarotation)

In somewhat stronger alkali the initial sugar is gradually converted into a mixture of all of the members of that series (Lobry de Bruyn phenomenon). d-Glucose, for example, gives a mixture of six isomeric hexoses including d-glucose, d-mannose and d-fructose, which result, according to Nef, from the formation of 1–2, and 2–3 d-glucose dienols, the H and OH groups on the second and third carbon atoms being thereby rendered mobile. In moderately dilute alkali the carbon chain is however not split (147).

With stronger alkali, however, there takes place, besides the rearrangements above mentioned, very extensive disruption of the molecule and the formation of more than a hundred different substances (147). The hexoses split into *a*, formaldehyde and aldopentose (123); *b*, dioses and aldo tetroses; and *c*, two trioses, by the intermediate formation of 1–2, 2–3 and 3–4 hexose dienols, and their spontaneous decomposition.



d – glucose di–enols

Nef and his pupils (75, 147, 187, 192) isolated from alkaline sugar solutions products of each type of decomposition, though there is reason to believe that the split into three carbon fragments is quantitatively

the predominant reaction, and that the single carbon methylene dissociation is very slight (unless oxidation occurs). The products formed by the splitting of the dienols are very unstable and reactive substances. In the absence of oxygen they form salts and polymerize to sugar resins or undergo an internal Cannizzaro rearrangement with the irreversible formation of "saccharinic acids," of which lactic acid is quantitatively the most important (147 d). *But in the presence of abundant oxygen the "dissociation products" are oxidized and neither resins nor saccharinic acids (lactic) are formed.*

This behavior of glucose in alkaline solution must be of the greatest importance to an understanding of the course of its breakdown in living cells, since there are very striking similarities in the reactions under the two conditions (200). In the test tube glucose is quite inert toward oxygen until its molecule is opened up by the action of alkali and converted into other very unstable and easily oxidizable substances; and in the animal body it is likewise altogether resistant to oxidation unless a combination of agencies, among which is the action of a pancreas hormone, converts it into reactive fragments whose identity remains unknown. Whatever the reactive derivatives may prove to be, whether formed by alkali in the test tube, or by biochemical agencies in the cells of the body, they are in both cases converted into the same substance, lactic acid, unless they are first oxidized. Lactic acid is thus the chief product of the anerobic catabolism of glucose, alike in cells and in vitro, and a knowledge of the unstable derivatives of glucose which are precursors of lactic acid is fundamental to an understanding of glucose metabolism.

The formation of lactic and other acids from sugars by the action of strong alkali, discovered by Hoppe-Seyler (94) has been studied by many workers (Kiliani (104), Buchner and Meisenheimer (15), Meisenheimer (140), Nef (147), Framm (65), Upson (192), M. Oppenheimer (159) and the quantitative yield has been reinvestigated recently in the writer's laboratory by T. E. Friedemann (66).

When warmed or allowed to stand in an excess of 0.2 to 1. N sodium or potassium hydroxide each molecule of glucose forms a total of about 1.6 molecules of (mono-basic) acids of which about one-half is lactic acid, very small amounts of CO_2 and formic, and the rest, non volatile ether-insoluble "saccharinic acids" (140, 158, 66). The maximum yield of lactic acid corresponds to slightly less than one molecule from each molecule of glucose, which suggests that the main dissociation is into

two 3-carbon fragments, only one of which in the test tube is converted into lactic acid.[1]

The main initial products are probably glyceric aldehyde or di-hydroxy acetone, formed by splitting of a 3–4 di-enol, half of which is transformed by loss and addition of water into methyl glyoxal, which is known to be immediately and completely converted by alkali into lactic acid (33). This interpretation of the course of the reaction is based upon the facts, *1*, that glyceric aldehyde and di-hydroxy acetone give, when acted upon by alkali, the same amount of total acids

TABLE 1

The action of alkali on glucose, glyceric aldehyde and glycol aldehyde: alkali "dissociation", with subsequent and simultaneous oxidation by hydrogen peroxide (T. E. Friedemann)

TREATMENT	SUGAR	TOTAL ACID	LACTIC ACID	FORMIC ACID	OXALIC ACID (AFTER OXIDATION BY ALKALINE $KMnO_4$)
		cc. N	*mols.*	*mols.*	
"Dissociation" by alkali	1 mol glucose	1.6	0.8	0.09	1.8
	2 mols glyceric aldehyde	1.5	0.8		1.8
	3 mols glycol aldehyde	1.45	0.6		1.8
"Dissociation" *followed* by oxidation by H_2O_2.	1 mol glucose	2.2	0.8	0.5	1.6
	2 mols glyceric aldehyde	2.55			
	3 mols glycollic aldehyde	2.1			
Simultaneous "dissociation" and oxidation	1 mols glucose	5.0	0	4.0	0.8
	2 mols glyceric aldehyde	5.8	0	5.6	0.09
	3 mols glycol aldehyde	5.7	0	5.4	
Untreated	1 mol glucose				3.0

and of lactic acid, as does glucose (158, 66) and *2*, that methyl glyoxal may be detected among the products formed from glucose by the action of alkali. Pinkus (167), Nef (147), Neuberg and Oertel,

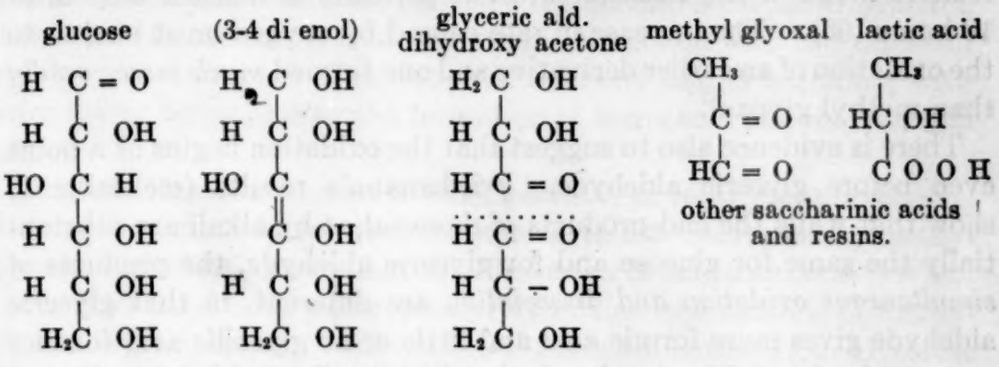
[1] The same inference can be drawn from the amount of oxalic acid formed by oxidation with alkaline permanganate. When oxidized before "dissociation" the 6-carbon chain of glucose yields 3 molecules of oxalic acid, while if oxidized by $KMnO_4$ *after* dissociation by alkali, slightly less than 2 molecules of oxalic are obtained, one from each 3 carbon fragment (see tables).

Although with alkali the maximum of lactic acid corresponds to only one half the carbon of glucose, the conversion in the animal body is believed to be complete.

Windaus and Knoop (194), Dakin and Dudley (26), Fernbach and Schoen (58).

In the accompanying table are given some results obtained by T. E. Friedemann which show that the amounts of total acids and of lactic acid from the alkali dissociation of glyceric aldehyde, and indeed of glycol aldehyde,[2] are almost identical with those of glucose.

It appears, therefore, that in half normal alkali and in the absence of oxygen, the dominant decomposition of glucose is:



Wohl (199) has suggested a slightly different course with the formation of methyl glyoxal glyceric aldehyde aldol, which then splits to 3-carbon fragments.

The *oxidation* of glucose in alkaline solution takes a different course from that outlined above. It has already been noted that the oxidation is *not* by way of lactic acid, but takes place *before* lactic acid is formed. If directly oxidized by hydrogen peroxide in alkaline solution, glucose yields no lactic acid, but 4 molecules of formic acid and 1 of glycollic acid (66); but if first dissociated by alkali the lactic acid formed is not oxidized by subsequent treatment with alkali (see table 1). Which of the precursors of lactic acid is the starting point of glucose oxidation in alkaline solutions? The following facts appear to indicate that it is not methyl glyoxal but a still earlier derivative.

[2] The fact that 3 molecules of the two-carbon glycol aldehyde when treated with alkali form nearly as much lactic acid as one molecule of glucose or two of glyceric aldehyde is additional evidence of the polymerization of sugars long known to take place in alkaline solution (16), (126), (59).

Evidently the glycol aldehyde is in large part condensed to tetrose or hexose which then dissociates into three-carbon fragments; and this type of reaction illustrates the processes involved in the formation of glucose (and lactic acid) from the derivative of glycine in the body.

The conversion of methyl glyoxal to lactic acid in alkaline solution is almost instantaneous, far more rapid than the rate of lactic acid production from glucose. The rate of lactic acid production is, therefore, not limited by the rate of formation of methyl glyoxal but by earlier reactions. But the rate of glucose decomposition is markedly increased hy oxygen (65). Judged by total acid production, loss of optical activity, or loss of reducing power, the reaction is practically complete in 0.5N KOH and H_2O_2 at 37°C. in 4 hours, while the same relative stage in the reaction without peroxide is reached only after 16 hours (66). The increase in rate caused by oxygen must be due to the oxidation of an earlier derivative and one formed *much more rapidly* than methyl glyoxal.

There is evidence also to suggest that the oxidation begins at a point even before glyceric aldehyde. Friedemann's results (see table 1) show that while the end-products of *dissociation* by alkali are substantially the same for glucose and for glyceric aldehyde, the products of *simultaneous oxidation and dissociation* are different, in that glyceric aldehyde gives more formic acid and little or no glycollic acid (oxalic) compared with 0.8 molecule of glycollic (oxalic) acid from glucose. This difference suggests that in spite of the fact that *alkali dissociation* gives the same products, the starting points of *oxidation* are different. This can only mean that in the main, the glucose derivative which is oxidized is *before* the stage of glyceric aldehyde.

That glucose itself is not directly oxidized is shown by an initial lag in the reaction, during which period some transformation is doubtless taking place; furthermore, by direct oxidation of glucose, the first product would be gluconic acid, which is not formed. We are thus left with the notion that oxidation begins with some intermediate between glucose and glyceric aldehyde. There appears to be no clear evidence as to what this intermediate may be, though we may recall the conceptions of Nef (147) and of W. Löb (123) that single carbon fragments are progressively split off from 1-2 di-enols (Nef, "Hydroxy methylene dissociation") and it may be imagined that the removal of these fragments by oxidation diverts the reaction from the otherwise predominating formation of 3-4 di-enols and their splitting to trioses. Or it may be supposed that the 1-2 dienols (γ-glucose?) are oxidized through the stage of the corresponding osones.

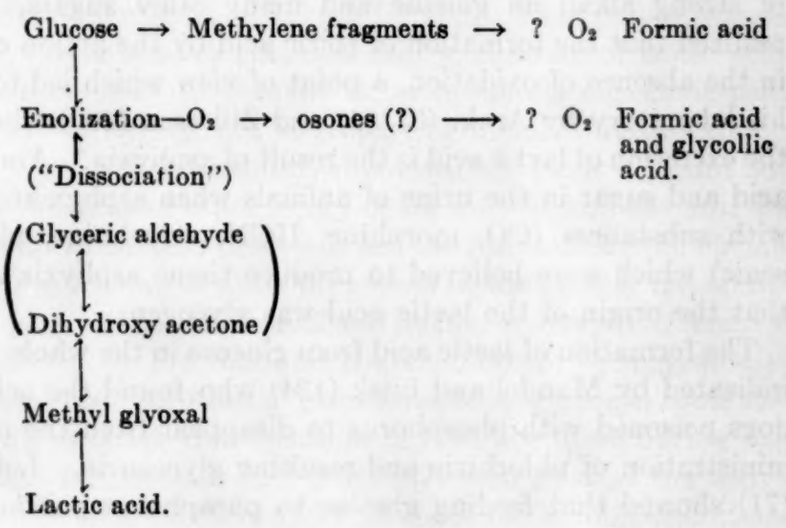
According to W. Löb and Beysel (123), and Witzemann (198), phosphates accelerate the oxidation by H_2O_2 even at very low alkalinity (at which reaction lactic acid formation does not occur). Experiments

by Harden and Henley (81) lead them to question whether the action is specific, but in view of the possible similarity of this *in vitro* effect of phosphates to their specific effect in yeast fermentation and in the formation of lactic acid in muscle, the subject merits more thorough investigation.

Although the exact identity of the first oxidizable derivative of glucose must be left undecided, there appears to be no escape from the conclusion that in alkaline solution oxidation begins at the early stages of dissociation and before the molecule is split into 3-carbon fragments. If this view is correct, it is of interest in connection with the question whether the main path of oxidation in the body has its beginning with a derivative *before* or *after* the formation of lactic acid and its 3-carbon precursors.

The reactions from glucose to lactic acid may be regarded as reversible. Although in alkaline solution lactic acid is not converted to methyl glyoxal, Dakin (26) shows that this does occur in acid solutions. When allowed to stand with p-nitrophenyl hydrazine, the osazone of methyl glyoxal is precipitated. The in vitro synthesis of hexose from glycerose is established by the well known work of Fischer. The conversion of methyl glyoxal to triose has not been observed in vitro, but the transformation of methyl glyoxal (26) as well as of lactic acid (26), (134) and glyceric aldehyde (177), to glucose is known to take place in the body.

The predominating reactions in the decomposition of glucose and its oxidation in alkaline solution may be represented in outline as follows. Besides the steps indicated, there are other dissociations and polymerizations, details of which are less well known.


Glucose → Methylene fragments → ? O_2 Formic acid
Enolization—O_2 → osones (?) ⟶ ? O_2 Formic acid and glycollic acid.
("Dissociation")
Glyceric aldehyde
Dihydroxy acetone
Methyl glyoxal
Lactic acid.

THE REVERSIBLE CONVERSION, GLUCOSE⟷LACTIC ACID IN THE BODY. The belief that lactic acid, (the presence of which in muscle was discovered by Berzelius), has its origin in carbohydrate was expressed by Liebig in 1847 (121), who formulated the view that sugar is converted in the blood into lactic acid which is oxidized as fast as formed, except where oxygen is lacking. Ever since that time lactic acid has been associated with muscular contraction, and the work of Du Bois Reymond, of Nasse and others clearly pointed to glycogen as its source. The evidence was reviewed by Nasse in 1879 (146) with the conclusion that the source of the lactic acid in muscle is, directly or indirectly, glycogen, which during contraction or in rigor is converted by a "spaltungsprocess" and not by oxidation, into sugar and lactic acid with the evolution of heat and work.

Although the methods used in the earlier investigations are, according to present standards, open to question and the reliability of the analytical results scarcely justified the conclusions, it is of interest to note that after a period of doubt as to the origin of lactic acid, the most modern view has returned to the early conception. There is no longer question that lactic acid of muscle is formed from glucose or glycogen, though the search continues for the unstable intermediate. Hermann's "inogen" has been abandoned, only to be replaced by Embden's "lactacidogen."

The close relation of lactic acid to carbohydrates was early indicated by its formation from sugar by fermentation (the inactive acid), and was greatly strengthened by the discovery of Hoppe-Seyler (94) already referred to that large amounts of this acid are formed by the action of strong alkali on glucose and many other sugars. Hoppe-Seyler realized that the formation of lactic acid by the action of alkali occurs in the absence of oxidation, a point of view which led to later work in his laboratory by Araki (3), (4) and Zillessen (202) who showed that the excretion of lactic acid is the result of asphyxia. Araki found lactic acid and sugar in the urine of animals when asphyxiated or poisoned with substances (CO, morphine, HCN, strychnine, phosphorus, arsenic) which were believed to produce tissue asphyxia and concluded that the origin of the lactic acid was glycogen.

The formation of lactic acid from glucose in the whole body was also indicated by Mandel and Lusk (134) who found the acid excreted by dogs poisoned with phosphorus to disappear from the urine after administration of phlorhizin and resulting glycosuria. Later Von Fürth (71) showed that feeding glucose to phosphorus poisoned rabbits in-

creased the lactic acid excretion, while the previous removal of glycogen reserves prevented a lactic acid excretion which otherwise resulted on exposure to cold (72). Similarly Sass (179) found that strychnine convulsions in depancreatized dogs produces less change in blood alkalinity (due to less formation of lactic acid) than in normal dogs.

The reverse transformation, lactic acid → glucose was demonstrated by Lusk and Mandel (134) and by Embden and Salomon (57) to occur when lactic acid is fed to phlorhizinized and depancreatized dogs. Under these conditions the conversion is believed to be quantitative, two molecules of lactic acid giving one of glucose. It may therefore be concluded that the animal body readily accomplishes the conversion glucose ⟷ lactic acid, in both directions; and it has been shown that the same reactions occur in isolated surviving tissues, muscle, liver, kidney and blood.

Muscle. Representing not only the largest single fraction of body substance, but the tissue for the performance of mechanical work, the greater part of carbohydrate metabolism occurs in muscle; and lactic acid is the agent through which the energy of glucose is transformed into heat and the work done by muscular contraction. The notable recent developments in knowledge of the mechanism of muscular contraction and of its energy conversion have been ably reviewed by A. V. Hill (87) in these REVIEWS and we shall mention only briefly the more important work bearing upon the source and fate of lactic acid.

Fletcher and Hopkins in 1907 (63) showed that in the freshly excised resting frog muscle the amount of lactic acid is small, but gradually increases, under *anerobic* conditions, as long as irritability remains. When exposed to oxygen lactic acid is not formed. As a result of stimulation, heating, mechanical or chemical injury, the rate of lactic acid production is enormously increased. After accumulating lactic acid in an atmosphere of hydrogen a large part of the acid disappears on exposure to oxygen. This "oxidative removal" of lactic acid did not take place after hashing or other injury of the muscle, and the authors concluded that the oxidation process is not the result of simple chemical reaction but is dependent upon the events of the normal life of the muscle. The source of the lactic acid and its fate on oxidative removal were not considered by Fletcher and Hopkins, except to suggest that the amount formed is "quite out of proportion to its glycogen content."

From an analysis of the time relations of heat liberation during and following muscle contraction, Hill (83), (87) showed that the *initial* rapid heat production is the same under anerobic and aerobic conditions

thus indicating that oxidation is not concerned with the act of contraction during which lactic acid is formed. In the absence of oxygen the lactic acid persists and there is no further (or a small) heat production; but in the *presence* of oxygen, the lactic acid disappears during the recovery phase following contraction, with a simultaneous liberation of heat. *The formation of lactic acid in muscle is therefore a very rapid process taking place in the absence of oxygen and without the liberation of much heat, and it is during the removal of the lactic acid that oxygen is consumed, heat liberated, and the greater part of the oxidation occurs.* It seemed probable that the lactic acid was oxidized (163), though Hill found the total heat evolved was sufficient to account for only about one-fourth the lactic acid which disappeared and its fate was therefore doubtful.

In 1911 Fletcher (62) reinvestigated the formation of lactic acid during autolysis of hashed muscle preserved with chloroform water and toluol, under which conditions a number of workers (143), (103), (174), (97) had observed a continued production of lactic acid to occur. Fletcher found with mammalian muscle a rapid production during the process of hashing and that little or no further increase occurred on autolysis with antiseptics unless the preparations became contaminated with bacteria. He was unable to obtain any increase of lactic acid on the addition of glucose or glycogen and concluded that the results of Ransom (168) as well as of Stocklasa (188) who claimed a fermentation of glucose by muscle and other tissues, with the formation of lactic acid, CO_2 and alcohol, were erroneous and probably due to bacterial action. According to Fletcher, there was no "glycolytic" formation of lactic acid in muscle, it being probably derived from some other "specific unstable precursory material" the amount of which is determined by "previous intra-cellular events."

A similar opinion was expressed by Embden, Kalberlah and Engel (49) who found that muscle press juice, prepared from frozen dog muscle, (and containing at the start almost a maximum of lactic acid) showed a further slight but distinct increase in lactic acid on incubation for one hour. The maximum increase of lactic acid was attained within 30 minutes, was favored by the addition of sodium bicarbonate, and was wholly inhibited by acid (105). Since added glucose or glycogen were not converted to lactic acid Embden and collaborators believed that their results excluded direct relationship between lactic acid and carbohydrate, and that the precursor must be some other unknown substance which they termed "lactacidogen."

Brief mention may be made at this point of the interesting investigations which have grown out of the discovery of "lactacidogen,"—an adequate account would carry us beyond the bounds of this paper. For a review see E. Schmitz (181). Following the observation of Embden, Griesbach and Schmitz (46) that an approximately equimolecular increase of inorganic phosphate accompanies the formation of lactic acid in muscle juice, and that added hexose phosphate led to an increase of lactic acid, it was concluded that "lactacidogen" is a hexose phosphate, and Embden and Laquer (51), (52) isolated from muscle an osazone identical with that of hexose phosphoric acid previously isolated from yeast by Lebedew (113) and Young. Embden, Schmitz and Meincke (55) confirm the parallel between lactic and phosphoric acid formation in dog and rabbit muscle and find that food has little influence on the amount of "lactacidogen." Even fasting and administration of phlorhizin cause no decrease, though a decrease is observed in muscles of phlorhizinized dogs after strychnine convulsions. In accord with their conclusion that "lactacidogen" is the "Tätigkeitsubstanz" of striated muscle, an increase of muscular power is claimed to result from the ingestion of sodium phosphate (44) and an increased urinary excretion of phosphate is observed a few hours after exercise (43). The amount of organic (lactacidogen) phosphate has been determined in various muscles of different species (20), (21), (22), (40), (1), (2), (130), (160), (193), in relation to muscular activity. A few reports have even appeared concerning therapeutic results with sodium phosphate, its use being suggested by the work of Embden and his co-workers (cited in (181)).

It appears to be established that phosphoric acid is intimately concerned with lactic acid production in muscle, and with muscular contration. In the form of "lactacidogen" it is doubtless a component of a precursor of lactic acid, though the first product may be methyl glyoxal. And if the view be correct that glucose oxidation takes place via lactic acid, phosphoric acid is equally concerned with carbohydrate metabolism. This is the view of Neuberg (152), Embden (39), Hopkins (93) and others. The fact, however, that the "lactacidogen" content of muscles, according to Embden, bears no relation to diet and therefore to the rate of glucose combustion, suggests caution in adopting the belief that it is concerned with the *oxidation* of carbohydrate.

Returning to the question of the ultimate origin of lactic acid in muscle, Laquer (111) showed that the "acid maximum" is self limited by its own acidity, and that when suspended in bicarbonate or phosphate

solutions, frog muscle at 30°C. forms lactic acid from added glucose as well as glycogen, while at 45°C. only glycogen and hexose phosphate give rise to lactic acid. After repeated freezing in liquid air the power to convert glucose, fructose, mannose, sorbose and maltose to lactic acid is lost, though a formation of acid from glycogen or hexose phosphate may be retained. The precursor is therefore not glucose or any of the other sugars mentioned, but is some other reactive form, available from glycogen, and into which glucose must be transformed before it can give rise to hexose phosphate and lactic acid (111), (112). According to these results the most sensitive stage of the reactions between glucose and lactic acid—the stage which is first lost—is the conversion of glucose to some active form related to glycogen, rather than the later stages of the reactions. It will be recalled that evidence has been cited which indicates that it is an early stage of glucose transformation which is lacking in diabetes.

In a series of very skilful investigations, Meyerhof (141) has proved that, when suspended in disodium phosphate solution, frog muscle, in an atmosphere of hydrogen, may convert nearly all of its glycogen and lower carbohydrate into lactic acid. He finds close agreement between the amount of total glucose which disappears and of lactic acid formed. The addition to chopped muscle of hexose phosphate, glycogen, glucose and other hexoses does not increase the *rate* of lactic acid formation, but when added at a time when the muscle carbohydrate has been used up, additional lactic acid is formed unless the properties of the living tissues are too far destroyed. The conversion takes place not by an autolytic process but by a continuation of the "vitalen Stoffwechsels," and while this continues glycogen and hexose phosphate are not superior to glucose.

More remarkable than the proof of the origin of lactic acid is the discovery by Meyerhof that the fate of the lactic acid, shown by Fletcher and Hopkins to disappear when the muscle is exposed to oxygen (oxidative removal), is, chiefly, *its conversion to glycogen*. This has been confirmed by Foster and Moyle (64) in Hopkins' laboratory. On repeating the experiments of Parnas who found that the amount of oxygen absorbed during oxidative recovery was sufficient to oxidize all of the lactic acid, Meyerhof (141) showed that the oxygen absorption could account for the oxidation of only one-fourth of the acid which disappeared. Furthermore, he finds, in confirmation of Hill, that the heat evolved during contraction and recovery amounts to about 900 calories for 1 gram of lactic acid formed and removed, an

amount which corresponds with the heat of combustion of $\frac{1}{4}$ of 1 gram of lactic acid (or of glucose or glycogen). Meyerhof, therefore, concludes that the chemical reaction for the burning of a glucose molecule in muscle may be written as follows.

I. Anoxidative phase

$$\underset{\text{glycogen}}{5/n\ (C_6H_{10}O_5)_n} + 5\ H_2O \rightarrow \underset{\text{glucose}}{5\ C_6H_{12}O_6} \rightarrow \underset{\text{glucose}}{1\ C_6H_{12}O_6} + \underset{\text{lactic acid}}{8\ C_3H_6O_3}$$

II. Oxidative phase

$$1\ C_6H_{12}O_6 + 8\ C_3H_6O_3 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 4\ C_6H_{12}O_6 \rightarrow 6\ CO_2 + 10\ H_2O + 4/n\ (C_6H_{10}O_5)_n$$

Four molecules of glucose are converted to 8 molecules of lactic acid and back again to glycogen, with the simultaneous combustion of 1 molecule of glucose, to furnish the energy needed for the transformation. Whether it is actually glucose which is burned or some of the lactic acid, Meyerhof leaves undecided. But his results appear to prove that the fate of the greater part of the lactic acid is *not oxidation*, but conversion to glucose or glycogen; and the idea conveyed by his equation is that *all* of the lactic acid is so converted, the heat being formed by the oxidation of glucose. This is a very fundamental point, for if *all* of the lactic acid formed be reconverted to glucose, *lactic acid would not be a stage in the path of glucose oxidation* in muscle. Meyerhof finds that the ratio of total lactic acid which disappears, to extra heat evolved or oxygen absorbed corresponds to 4 molecules disappearing: 1 molecule oxidized. Hartree and Hill (83) find ratios from 4.9:1 to 6:1. Oxygen is essential to the disappearance of the lactic acid but at most only 1 out of 4 or 6 molecules can be oxidized, the rest reappearing as glycogen or glucose. In spite of oxidation (and only in the presence of oxygen) the *predominant* reaction is the formation of glycogen, which if it occur via triose, *does not involve oxidation*. It appears to be a very paradoxical situation. The course of the reactions is not established and their mechanism is wholly unknown. It is referred to by Embden (47, p. 143), Parnas (163) and Meyerhof as a "coupled reaction" by which the lactic acid is converted to glycogen at the expense of energy derived from the oxidation of a common intermediate—lactic acid or glucose or perhaps of other substance. Hill appears to regard lactic acid as the substance oxidized (83), and with Lupton (88) has recently reported very interesting studies of the relation of lactic acid formation and its oxidation to muscular work and efficiency in man. Perhaps the following may be considered among the possibilities.

1. The reconversion (via triose) of three-fourths to five-sixths of the lactic acid to glucose and the simultaneous oxidation of the remainder (via pyruvic acid) to CO_2, by a "coupled reaction" (83), (141). How the oxidation could facilitate the conversion of lactic acid to glucose is not evident. It is perhaps conceivable that it may result from local change in hydrogen ion concentration. An alkaline reaction appears to favor lactic acid formation, and acid to hinder or limit it.

2. The reconversion of *all* of the lactic acid to glucose or glycogen, with the simultaneous oxidation of glucose or a derivative, (or of fat or other substance).

3. The oxidation of the whole of the lactic acid via pyruvic acid, acetic aldehyde, and CO_2, the acetic aldehyde being converted to glucose. This is perhaps less probable, since it would account for the conversion of only two-thirds of the carbon to glucose and one third to CO_2, corresponding to a ratio of lactic acid disappearing: oxidized, of 3:1 instead of 4:1 or 6:1. Furthermore the evidence is conflicting as to whether acetic aldehyde is convertible into glucose in the body. Another route, via glyceric acid and glycol aldehyde has been suggested (165). The direct oxidation of lactic acid will be considered again in a later section.

Blood and kidney tissue. There is clear evidence that the transformation of glucose to lactic acid takes place also in the blood, the kidney and the liver as well as in muscle; and it is probable that the reaction may take place wherever glucose is metabolized. In 1877 Spiro found lactic acid in blood (186). Levene and Meyer (115) showed that sterile leucocytes suspended in phosphate solution convert added glucose into d-lactic acid. Although the amount of lactic acid formed was less than the amount of glucose which had disappeared, later experiments indicated no evidence of oxidation. Dilution with water, or the presence of toluol prevented the formation of lactic acid.

The amount of lactic acid in whole blood increases on standing (70) and with the increase in lactic acid there has been observed a corresponding decrease in glucose (106), (107). This conversion of blood sugar into d-lactic acid appears to be the explanation of blood "glycolysis" (131) and is brought about by the cells and not by plasma (156). Sterile kidney tissue in phosphate solution and in absence of antiseptics, converts glucose into d-lactic acid, but in the presence of toluol the conversion does not take place (117).

Liver perfusion. Much valuable evidence on the reactions between glucose and lactic acid has resulted from the artificial perfusion of

excised liver by a technique developed especially by Embden. By this method of investigation also it has been demonstrated that the reaction glucose $\rightleftarrows$ lactic acid may proceed in either direction under different circumstances.

With livers containing glycogen there is an increase of glucose (108), and of lactic acid (38), (50) in the perfusion fluid. But with livers rendered glycogen-free by producing strychnine convulsions in the animals, lactic acid is not formed, unless glucose, fructose, alanine, or glycerol is added to the blood. The addition of arabinose or inosite is without effect (50), (53). These results show that glycogen or glucose is the source of the lactic acid.

The reverse transformation, lactic acid to glucose, takes place on perfusing glycogen-free livers, either normal or diabetic. Embden (38) found an increase of blood sugar with livers rendered glycogen-free by strychnine convulsions, while Embden and Kraus (50) found under the same conditions a decrease of lactic acid. And Embden and Isaac (48) showed that the loss of lactic acid closely parallels the gain of glucose, thus indicating the source of the glucose and showing an approximately *quantitative conversion.*

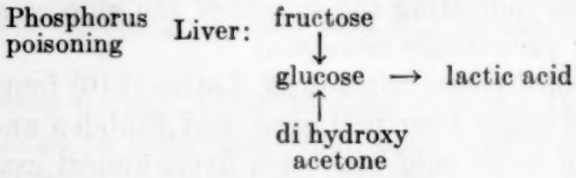
With livers of *depancreatized* dogs, Lattes (110) found a marked *increase* of blood sugar after perfusion, and Embden and Isaac showed that the loss of lactic acid with such livers almost exactly equals the gain of sugar, again indicating a quantitative conversion. Added glucose did *not* give rise to lactic acid with diabetic livers, although it is so converted by normal livers. Barrenscheen (10) reported that diabetic livers on perfusion do not form glycogen from added hexoses, under conditions in which glycogen is formed by the normal liver. These results appear to show that the liver of the normal dog, if glycogen be abundant, converts it to glucose and to lactic acid; or when the glycogen store is low, it can reverse the process and form glycogen from added glucose or lactic acid. But the liver of the diabetic dog has lost the power to carry the reactions, glucose to lactic acid, and glucose to glycogen, but retains the power to convert lactic acid to glucose. The conditions may be represented as follows:

Normal liver: Glycogen $\longleftrightarrow$ glucose $\longleftrightarrow$ lactic acid
Diabetic liver: Glycogen $\longrightarrow$ glucose $\longleftarrow$ lactic acid

These findings recall the facts that the diabetic animal or human subject converts lactic acid and many other substances into glucose, though he does not readily form lactic acid nor store glycogen. Wood-

yatt (201) reports that the muscle of a human diabetic does not develop lactic acid in rigor, as does normal muscle. Similar, though less striking results have been reported by Von Furth (73). But Forschbach (63a) found that the muscle of a depancreatized dog contained a normal amount of lactic acid. And Parnas (164) finds that the muscle of depancreatized frogs does not differ from normal frog muscle in its formation and oxidation of lactic acid on contraction, and suggests that diabetes does not interfere with the consumption of carbohydrate for muscular work. This conclusion can hardly be accepted for man or warm blooded animals.

The livers of animals poisoned with phosphorus, in contrast with diabetic livers, on perfusion are found to possess the power to form lactic acid from glucose or fructose, but have lost the power to accomplish the reverse change of lactic acid to glucose. They do however form glucose from dihydroxy acetone (101). The defect thus would appear to between lactic acid and triose. Fructose is converted to glucose by phosphorus livers as well as by normal livers.



Livers of animals poisoned with both phosphorus and phlorhizin form no glucose from lactic acid. This result is surprising in view of the fact that phlorhizin injection (and glycosuria) inhibits lactic acid excretion by phosphorus poisoned animals (134). E. Neubauer (148) claims that rabbits poisoned with phosphorus can form glycogen from fructose but not from glucose.

THE INTERMEDIATE PRECURSORS OF GLUCOSE $\rightleftarrows$ LACTIC ACID IN VIVO. The evidence cited shows that each of the above-named substances may be converted into the other, in the body as a whole and in isolated organs or tissues. The conversion is the result of a series of transformations, the analysis of which has been attempted by determining what possible intermediates or related substances give rise to either glucose or lactic acid when fed to animals or when added to isolated organs or tissues or to perfusion fluids. The following facts appear to indicate the same intermediates in the *in vivo* reaction as in the *in vitro* formation of lactic acid from glucose by alkali.

d-l Glyceric aldehyde may be oxidized in the body (150), is destroyed by liver tissue (184), is converted into d-glucose when fed to phlorhizin-

ized dogs (177), forms glycogen on perfusion of tortoise livers (162), forms d-glucose and d-sorbose on perfusion of dog liver (56), and is converted into d-l and l-lactic acid by washed blood corpuscles and by perfusion of livers of fasting dogs (42). It may therefore go in either direction: glucose ← glyceric aldehyde → lactic acid. Similarly, dihydroxy acetone is converted to d-glucose when injected into phlorhizinized dogs (172), and by perfusion of dog liver it gives both d-glucose (56) and d-l lactic acid (42). On standing with washed blood cells of the dog it is converted to d-l lactic acid less readily than is d-l glyceric aldehyde, but pig blood corpuscles form from it d-lactic acid (125), (78).

Methyl glyoxal is likewise converted into glucose by the phlorhizinized diabetic dog (30) and into d-l-lactic acid by many hashed tissues (by the enzyme "glyoxalase"), (28), (29), (151a), on liver perfusion and by sterile leucocytes and kidney tissue (116).

As emphasized especially by Dakin and Dudley, Levene and by Neuberg, methyl glyoxal must, in the light of the above facts, be regarded as an intermediate between glucose and lactic acid. In view of the wide occurrence and great activity of the enzyme "glyoxalase" there is good reason to believe that it is the immediate precursor of lactic acid in vivo, as it doubtless is in the formation of lactic acid from sugar *in vitro*. Dakin has pointed out that the undoubted conversion of l-lactic acid into d-glucose seems to necessitate its passage through an optically inactive intermediate, and that this is probably methyl glyoxal or dihydroxy acetone. The opposite opinion advanced by Embden, Baldes and Schmitz (42) that methyl glyoxal cannot be an intermediate because its molecule contains no asymmetric carbon atom, can scarcely be accepted in view of the wide distribution of "glyoxalase" and of the undoubted conversion of l-lactic acid and of many recemic compounds into d-glucose. Neuberg (152) points to the possible existence of forms of methyl glyoxal with optically active carbon atoms, and suggests schemes by which molecular asymmetry might be conferred by it as an intermediate.

There is therefore no obstacle to the belief that the three substances, glyceric aldehyde, dihydroxy acetone and methyl glyoxal (together with the hexose phosphate, "lactacidogen") represent the main intermediates between glycogen or glucose and lactic acid.

Although the reactions in the direction of lactic acid have close analogies in the *in vitro* behavior of glucose, the reverse reactions, lactic acid to glucose, which appear to occur only under the influence of living cells and *in the presence of oxygen*, are difficult to visualize.

The apparent necessity of oxygen suggests that the path may be by way of an oxidation of lactic acid. Such a conception has been advanced by Parnas and Baer (165) who propose a series of reactions by which three molecules of lactic acid yield progressively glyceric acid, β-hydroxy pyruvic acid and glycol aldehyde, the latter condensing to a molecule of glucose. As evidence for their scheme they find an increase of glycogen in tortoise liver after perfusion with glycol aldehyde (but not with glycollic or glyoxylic acids) and a conversion of glyceric acid, lactic acid, glycol, and glycol aldehyde into glucose by phlorhizinized rabbits. Barrenscheen also (10) observed the formation of glucose from glycol aldehyde, glycerol, glyceric acid and lactic acid on perfusion of livers of phlorhizinized dogs. However, Baldes and Silberstein (8) in similar experiments were unable to show the formation of sugar from glyceric acid or glycol aldehyde, and the scheme of Parnas and Baer (165) has not been generally accepted (27, p. 116). It appears, nevertheless, that the two-carbon glycol aldehyde, if formed in the body, may be converted into glucose. Although the observation by Mayer (136) of glycosuria after its administration to rabbits may be of doubtful value the experiments of Sansum and Woodyatt (178), Cremer (25) and Greenwald (76) with phlorhizinized dogs, if not decisive, appear to show the formation of glucose from the injection of glycol aldehyde. Neither glyoxal nor glycollic acid, into which the former is converted by surviving liver (31), are converted to glucose (76). It seems, therefore, that glycol aldehyde can form glucose only by direct condensation before oxidation or action by "aldehydemutase" (161). It will be recalled that in alkaline solution glycol aldehyde polymerizes to tetroses (60) which then dissociate with the formation of lactic and other acids (66).

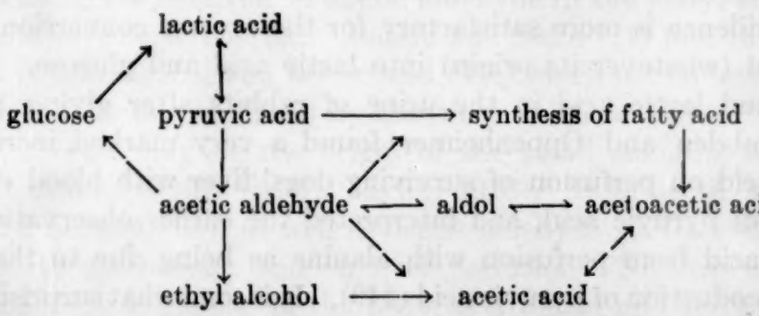
Another possible pathway of oxidative conversion of lactic acid to glucose, via pyruvic acid and acetic aldehyde, the latter condensing, with oxidation to glucose, has been already noted. (P. 414 see also p. 422.) Opposed to such explanations is the fact that diabetic animals appear to convert all of the carbon of lactic acid into glucose, and if this be true a direct conversion, without loss of carbon by oxidation, is of course demanded. Glycid and acetole are not convertible into glucose (77).

It appears at present that the most probable course from lactic acid to glucose is passage through almost if not quite the same intermediates which precede the formation of lactic acid, i.e., by a series of *reversible reactions;* though the apparent oxidative character of the reaction

suggests caution in the adoption of this conclusion. The step from lactic acid to methyl glyoxal has an in vitro parallel in the formation of methyl glyoxal in lactic acid solutions (30). The next step, from methyl glyoxal to triose has not yet been accomplished in vitro, but the third step, triose to hexose was demonstrated by the well known synthesis of fructose from glycerose by Fischer.

THE FURTHER FATE OF LACTIC ACID. Of the two possibilities for the disposal of lactic acid one has just been considered—its direct reconversion to glucose and glycogen via methyl glyoxal and triose. The other is its direct oxidation. The first seems very probably to occur, and the second, oxidation by way of pyruvic acid and acetic aldehyde, represents what appears to be the prevailing view as to the main path of carbohydrate oxidation (Neuberg, Embden, Meyerhof).

In 1911 Neuberg (153) discovered that yeasts decompose pyruvic acid into acetic aldehyde and CO_2; and in a series of later investigations with various collaborators he has admirably demonstrated the fundamental importance of these substances in the intermediary metabolism of glucose in fermentations. There has gradually been accumulated a train of evidence which has been interpreted in support of the idea that glucose metabolism in the animal body follows much the same path as in fermentation and that pyruvic acid and acetic aldehyde are in the animal body also obligate intermediates. These two substances are regarded not only as steps in the oxidation of glucose, and in the oxidative reformation of glucose from lactic acid, but as a bridge by which glucose passes to fatty acid or to acetoacetic acid, or vice versa. The following diagram represents the possible reactions under consideration.



Without questioning the experimental results the reviewer finds it difficult to accept some of these conclusions. Although on oxidation by hydrogen peroxide *in vitro*, lactic acid yields pyruvic acid (92) no *direct* evidence appears to be available as to the products of lactic acid oxidation in the body. That pyruvic acid is the product is inferred

from the fact that its decomposition product, acetic aldehyde has been found, and from the fact that the reverse reaction, its reduction to lactic, is established. Tschernorutzki (191) found that on digestion with muscle or liver hash pyruvic acid disappears, but he was unable to find acetic aldehyde. By liver perfusion, however, Embden, and Oppenheimer (53) observed on adding salts of pyruvic acid an increased formation of acetoacetic acid, which they interpreted as being due to preliminary formation of acetic aldehyde and its condensation to aldol. Friedmann had earlier found that an increase of acetoacetic acid resulted from perfusion of liver with acetic aldehyde. Although the amounts of acetone (from acetoacetic acid) found by Embden and Oppenheimer were neither large, nor constant, the authors put forward the view (53), (54) that glucose → lactic acid, → pyruvic acid, → acetaldehyde → acetoacetic acid, → acetic acid, is the main path of glucose metabolism.

If lactic acid (or glycogen) is the source of pyruvic acid and if the latter gives rise to acetic aldehyde, aldol and acetoacetic acid, as depicted in the diagram, it might be expected that with increasing formation of lactic acid there would be increasing amounts of acetone. But the reverse appears to be the case. On perfusion of glycogen-rich livers, (or normal glycogen-free livers with added glucose or fructose), there is much lactic acid formed, but little or no acetone; while with diabetic livers, which convert lactic acid almost quantitatively to glucose, large amount of acetoacetic acid appear (48). This would seem to prove that the source of the acetoacetic acid (and of pyruvic acid and acetic aldehyde) is not lactic acid. If lactic acid is oxidized its products must *inhibit* the formation of acetoacetic acid.

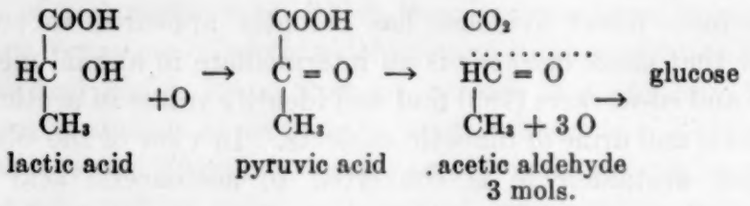
The evidence is more satisfactory for the reverse conversion of pyruvic acid (whatever its origin) into lactic acid and glucose. Mayer (137) found lactic acid in the urine of rabbits after giving pyruvic acid. Embden and Oppenheimer found a very marked increase of d-lactic acid on perfusion of surviving dogs' liver with blood containing salts of pyruvic acid, and interpreted the earlier observation (50) of lactic acid from perfusion with alanine as being due to the intermediate production of pyruvic acid (149). It is somewhat surprising that on perfusion of livers of phlorhizinized dogs Baldes and Silberstein (8) and Barrescheen (10) could find no evidence of glucose formation from pyruvic acid (or from analine or serine). When administered to phlorhizinized dogs, pyruvic acid is, in part at least, converted to glucose, presumably by reduction to lactic acid (169), (170), (32),

(25). This conversion is less constant and complete than is observed with lactic acid, and it is perhaps possible that this is to be explained by a dual path, lactic acid, ← pyruvic acid, → acetic aldehyde. Levene and Meyer (119) were unable to observe any action on pyruvic acid by sterile leucocytes, under conditions where a formation of lactic acid from glucose was found to take place.

Other more direct evidence has recently appeared in support of the view that *acetic aldehyde* is an intermediate in animal metabolism. Stepp and co-workers (189) find and identify traces of acetic aldehyde in the blood and urine of diabetic subjects. In view of the observation (67), that acetaldehyde is converted to acetoacetic acid on liver perfusion, they assume the aldehyde to be a precursor via aldol, of the "acetone bodies." But Fricke (69) was able to find only doubtful traces of aldol from 50 liters of diabetic urine. Hirsch (90) shows that on aerating hashed frog muscle and fish muscle in the presence of "dimedon," acetaldehyde is formed (73 mgm. from 500 grams muscle). Traces were obtained also from fresh frog muscle, the amount not being increased on aeration except in the presence of the "dimedon" so successfully used by Neuberg and Reinfurth (155) in yeast fermentation. The origin of the acetaldehyde in these experiments was supposed to be pyruvic acid, derived from the oxidation of lactic acid, and thus from carbohydrate, though direct evidence for the latter assumptions is lacking. It is perhaps equally probable that the source is pyruvic acid formed from amino-acid (149). Until a decision on these points can be reached, judgment as to the significance of these observations must be withheld.

Concerning the behavior of acetic aldehyde in the body, the following results have been reported. Batelli and Stern (11) find that tissues convert it into alcohol and acetic acid (41), which action according to Parnas (161) is accomplished by enzymes which he terms "aldehyde mutase." This may be the origin of the small amounts of alcohol found in animal tissues (A. E. Taylor, 1913). Both acetic acid (124) and ethyl alcohol (138) as well as acetic aldehyde (67) are claimed to give rise to acetoacetic acid on liver perfusion. Neither acetic acid nor alcohol are ketogenic in the body as a whole, and the above conclusions from liver perfusion are open to question. Neither acetic acid nor ethyl alcohol are convertible into glucose. On giving acetic aldehyde subcutaneously to phlorhizinized dogs, Ringer and Frankel (172) found an increased glucose excretion, although Sansum and Woodyatt (176) after similar experiments concluded that a formation of

glucose from acetic aldehyde does not take place. Lusk (128) states that S. R. Benedict has found the conversion of acetaldehyde to glucose to be complete in the phlorhizinized dog. If this be true, it perhaps constitutes another path for the oxidative conversion of lactic acid to glucose considered in an earlier section.



Ringer and Frankel found acetaldehyde to be antiketogenic, which accords with its conversion to glucose, but is contrary to its apparent conversion into acetoacetic acid on liver perfusion (67). Fricke (69) quotes Reizenstein as showing that acetaldehyde is not easily oxidized in the body, a considerable fraction being excreted in the urine after its administration. If this be true, it would seem unlikely that such large amounts of acetaldehyde are formed as must occur if it is an intermediate in glucose oxidation, or in the conversion of lactic acid to glucose.

Although a decision as to the origin of acetic aldehyde and the extent of its rôle in metabolism must await more conclusive evidence, it seems to the reviewer probable that it has no direct relation to glucose metabolism. If formed it would give rise, at least in part, to acetoacetic acid (67); but the oxidation of glucose *prevents* ketosis. In view of the definite and quantitative relations which appear to exist between the opposing metabolism of ketogenic and antiketogenic substances (Shaffer) (182) one is inclined to doubt whether any intermediate of antiketogenic glucose can *directly* give rise to ketogenic molecules, or vice versa. The conversion of carbohydrate into fat is, of course, an example of such transformation, but this presumably occurs only with carbohydrate plethora. Although on theoretical grounds one might expect the reverse transformation, there is no acceptable evidence that such actually occurs in the body. When the amount of carbohydrate available is small and the need for glucose correspondingly great, a transformation of ketogenic to antiketogenic substance in either direction does not appear to take place (182). It must be noted however that the opposite conclusion is reached in a recent review by Geelmuyden (74).

The argument that acetic aldehyde is formed in the animal body from glucose via lactic and pyruvic acids, is converted by synthesis back to glucose, is converted to acetoacetic acid or formed from it, and is thus a bridge between carbohydrate and fat, is a very attractive hypothesis, but one which the reviewer regards as fallacious.

The conversion of carbohydrate into fat. In connection with the rôle of pyruvic acid and acetic aldehyde, it should be noted that each of these substances has been regarded as the probable intermediate in the synthesis of fatty acid from glucose. Based on a suggestion of *Nencki*, Magnus-Levy (133) proposed a progressive aldol condensation of acetic aldehyde formed from *lactic acid*, followed by reduction of the β-carbon alcohol groups, as the sequence in the formation of straight chain fatty acids.

A somewhat similar hypothesis was developed by Smedley (185) who showed that a repeated condensation of pyruvic acid with loss of CO_2 and reduction may result in straight chain fatty acids with an even number of carbon atoms.

Some such process is probably the explanation of fatty acid synthesis, but in the absence of direct evidence, they can be regarded only as speculative hypotheses. No other scheme seems to have been proposed.

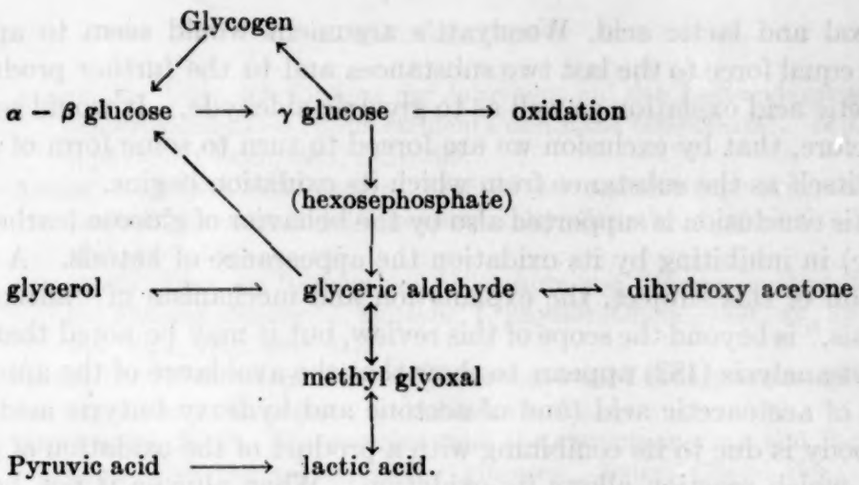
THE PATH OF GLUCOSE OXIDATION IN THE ANIMAL BODY. It has been noted above that there are difficulties in the acceptance of the view that glucose is *oxidized* via lactic and pyruvic acids and acetaldehyde. A summary of the main points opposing such a course may be made as follows.

Although the conversion, glucose ⇄ lactic acid, takes place in either direction in the body as a whole, or in isolated muscle or liver, the *preponderating* reaction in the presence of oxygen, seems to be in the direction, glucose ← lactic acid. Lactic acid appears only as the result of muscular activity or asphyxia, and when it disappears from isolated muscle or during liver perfusion, it is *chiefly* converted into glucose or glycogen. There is no convincing evidence of its direct oxidation to pyruvic acid or other products, though the possibility that such occurs cannot be denied. When pyruvic acid is introduced into the body or used in liver perfusion its *main* fate seems to be the conversion to glucose or lactic acid.

According to present views, the sudden, explosive formation of lactic acid is the essential agent causing muscular contraction (93), (87), (141). But during muscular work on low carbohydrate diets, the respiratory quotients indicate that the substance oxidized is fat. (See for example the careful work of Krogh and Lindhard (109) also Eckert (36).) Under such conditions presumably lactic acid is formed, but it cannot be the substance *burned:* unless it be supposed that fat, the ultimate source of the energy, is converted into glucose or other precursor of lactic acid in the course of oxidation. Although held by some authors, (see review by Geelmuyden) (74) the best evidence seems to be contrary to the latter view. If glucose or glycogen is the only source of lactic acid, and if the latter is necessary to muscular contraction its *conservation* under such circumstances would seem to be essential.

In the diabetic state, with the loss of the power to oxidize glucose, there is also the inability to burn lactic acid and any of its precursors or intermediates, proved by the fact that all are completely and quantitatively converted to glucose. The livers (48) and muscles (73), (201) of diabetic animals appear to have lost the power to form lactic acid from glucose which suggests that the diabetic defect is in *the formation of some lactic acid precursor from ordinary glucose,* and since lactic acid itself (or other intermediates) as well as glucose is not burned, one must suppose either an *additional* defect preventing lactic acid oxidation, or assume that it is not directly oxidized even by the normal, but must be first converted to glucose. The latter alternative is the simpler explanation. Recent evidence has been cited which seems to indicate that the diabetic defect is located in the step between ordinary α- and β-glucose and a tautomeric form, γ-glucose, which it may be imagined is an essential step in the transformations to glycogen, or to methyl glyoxal and lactic acid, or to oxidation.

This conception appears to remove another fundamental difficulty. According to the results cited from Embden's laboratory, the liver of the depancreatized dogs have lost the power to form lactic acid from glucose, but retain the ability to perform the reverse change, possessed alike by the normal and diabetic intact animal (48). It is difficult to interpret these results on the basis of a single reversible reaction the same throughout in both directions. But if the reactions be looked upon as in a cycle, at one stage of which the pancreatic hormone is necessary, the experimental findings become more nearly intelligible. The following crude outline represents this idea, and appears to the reviewer best to coincide with existing facts.



The diagram is intended to indicate that the synthesis of glucose from lactic acid and intermediates gives ordinary α-β-glucose, which must be transformed into a reactive isomeric (ethylene oxide?) formed by the intervention of pancreas hormone (9), (17), (34) before the reverse change to lactic acid can occur; and that the formation of lactic acid is a side reaction, asphyxial in character, and concerned with muscular contraction and perhaps with other phenomena, but probably not with glucose *oxidation*. Pyruvic acid is represented as undergoing reduction to lactic acid; the reverse reaction seems doubtful.

If lactic acid be rejected as the path of glucose oxidation, at what other intermediate does its oxidation start? The same sort of objections seem applicable to methyl glyoxal and to glyceric aldehyde. Both are converted into d-glucose by the diabetic organism, and this fact may be interpreted either by supposing the diabetic defect to prevent their direct oxidation, or that their conversion to glucose is the normal reaction, and that it is glucose which fails of further utilization. In view of the discovery by Dakin and Dudley (29) that pancreas extracts inhibit the conversion of glyoxals to hydroxy acids by other tissues, and the statement by Hopkins (93) that pancreas extract slows the rate of lactic acid production in hashed muscle, it is tempting to pick the very reactive methyl glyoxal as the substance oxidized.

An observation by Sansum and Woodyatt (178) casts doubt upon glyceric aldehyde. They find that the tolerance of d-glyceric aldehyde, or the rate at which it may be injected intravenously without triosuria, is only one-sixth the rate at which glucose is *burned* in the body, and that the combustion of glucose cannot, therefore, take place via glyceric aldehyde. Assuming glyceric aldehyde to be a precursor of methyl

glyoxal and lactic acid, Woodyatt's argument would seem to apply with equal force to the last two substances and to the further products of lactic acid oxidation as well as to glyceric aldehyde. It would seem, therefore, that by exclusion we are forced to turn to some form of glucose itself as the substance from which its oxidation begins.

This conclusion is supported also by the behavior of glucose (carbohydrate) in inhibiting by its oxidation the appearance of ketosis. A discussion of this subject, the explanation and mechanism of "antiketogenesis," is beyond the scope of this review, but it may be noted that its recent analysis (182) appears to show that the avoidance of the appearance of acetoacetic acid (and of acetone and hydroxy butyric acid) in the body is due to its combining with a product of the oxidation of glucose, which reaction allows its oxidation. When glucose is not being oxidized and its "ketolytic" oxidation product is not being formed in sufficient amounts, in relation to the rate of formation of acetoacetic acid, the latter accumulates with resulting ketosis. The oxidation of glucose in alkaline solution *in vitro* accomplishes the oxidation of acetoacetic acid in a manner which appears to be quite analogous to the reaction in the body. In the human subject the maximum antiketogenic effect of glucose corresponds to approximately 2 molecules of acetoacetic acid for 1 of glucose, which is also the ratio of glucose to keto-acid in the *in vitro* reaction. But *in vitro*, each molecule of glyceric aldehyde, glycol aldehyde (their oxidation products) or glyoxal likewise reacts with two of acetoacetic acid. If in the body, (or in vitro) glucose were first dissociated into two molecules of glyceric aldehyde, we should expect that under favorable conditions it would accomplish the oxidation of (2 × 2) or 4 molecules of keto-acid. But this would be *twice* the maximum antiketogenic effect of glucose which has so far been observed. According to this line of argument *it must therefore be concluded that the six carbon glucose is probably oxidized to a single molecule of ketolytic substance without previous splitting to triose.*

What the first oxidation products of glucose may be are quite unknown. Levene and Meyer could find no evidence that glucosone is acted upon by tissues (120). In view of the apparent similarity between its oxidation in the body and in alkaline solution *in vitro*, further study of the latter may be of assistance in solving the problems.

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BACTERIAL METABOLISM

ARTHUR ISAAC KENDALL

Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois

INTRODUCTION. "In the study of the microscopic forms known as bacteria we have what might fitly be called the focal point of the various branches of biological science. Though their investigation may require careful morphological researches, yet the unmistakable monotony of form, combined with a considerable variation of physiological activity, has compelled the bacteriologist to pay much attention to means by which such physiological variations may be more or less accurately registered in order that they may serve as a supplementary basis for classification. Again, with the unicellular organisms the manifestations of cell activity become the most important phenomena for study. These manifestations bring together the fields of physiology and chemistry and make Bacteriology in one sense a branch of physiological chemistry" (1).

Three decades have passed since this prophetic assertion of the interdependence and mutual relations of the fundamental dynamic biological sciences to the solution of the manifestations of cellular activity was published, yet even today its full import is foreshadowed rather than realized.

These three decades, nevertheless, have been replete with contributions to the well-being of mankind. They have witnessed the maturation of the germ theory of disease, the rise and development of a new science, immunology, and the first great accomplishments in the field of preventive medicine. The widening of the frontiers of knowledge in physiology and pathology have influenced profoundly the advance of curative medicine.

The pioneer work in the accurate measurement of the interchange of living cells with their environment belongs also to this period, and the widespread transition from the static or morphologic contemplation of biologic science to the dynamic or causative aspect has revealed new and fruitful fields for study.

Bacteriology also has passed through this transitional period from morphology and classification to an inquiring into the causation and effect of microbic activity. The search for curative serums, antitoxins, vaccines, and the response of the host to these remedial agents, has impelled investigators in many fields to take up anew the study of cellular activity and the results thereof. It is not without significance that many of the requisite procedures for this study have been developed by the biochemists.

PART I. THE BACTERIAL CELL. Bacteria are among the smallest of known living things. The phenomena of life are revealed in their lowest terms in this group of organisms. All of their vital functions are consummated in single cells, so minute that fully twenty-five thousand of average size, laid side by side, would scarcely span the distance of an inch. Bacteria possess no morphologically definable nucleus,[1] they are devoid of chlorophyll or other photodynamic pigment, and they reproduce by simple fission, the resulting individuals being of approximately equal size. This process of transverse fission of a single sexless unit is the simplest method of reproduction thus far revealed in nature. It may be very rapid. For example, cholera vibrios placed in a very favorable environment may divide individually into two daughter vibrios every fifteen minutes for limited periods of time. The *theoretical* twenty-four hour progeny of a single cholera vibrio, therefore (ninety-six generations), would be 2^{96}, or nearly 8×10^{28}, a truly prodigious number. Fortunately, many natural barriers, as mutual antagonism, exhaustion of food and accumulation of waste products, restrain the multiplication of the microbes after the first few hours' growth and keep the progeny within endurable limits. Nevertheless, the rapid increase of bacteria in short periods of time is no inconsiderable factor in determining the rate and extent of the changes they induce in their nutritive environment.

Another noteworthy characteristic of the bacterial cell is its disproportionately large ratio of surface area to weight or volume. Thus, a typhoid bacillus is a microbe of average size. Its volume is approximately 0.000,000,002 cc. and its weight about 0.000,000,002 mgm. (2). Its surface area, however, is nearly 0.000,01 sq. mm. In no other group of known living things, except possibly the filterable viruses, is this ratio of surface to volume exceeded, or even equalled.

[1] The bacterial cell, chemically considered, is relatively rich in nuclear substance, however.

Inasmuch as the energy requirements of living things in general are determined largely by the ratio of their surface to their volume, this large surface-volume ratio characteristic of bacteria furnishes a background for the well attested magnitude of microbic interchange with their nutritive environment, notwithstanding their minuteness. The two factors—rapid multiplication and large surface to volume ratio—underlie the phenomena of bacterial nutrition.

PART II. BACTERIAL METABOLISM. *a. General considerations:* Bacteria and animals differ from plants in that they possess no chlorophyll or other photodynamic pigment. Chlorophyll is a transformer of solar energy into growth energy. In virtue of this transformed energy the plant weaves the structural elements—nitrates, carbon dioxide, water and salts—into the complex proteins which form the living substance of the plant tissues. The architectural design of these organic complexes resides in the heredity of the plant itself, which guides the life elements into the complex specificity of the plant protoplasm.

The absence of chlorophyll or other photodynamic pigment from the bacterial substance makes it quite evident that the driving force of microbic existence must come directly or indirectly from energy-producing substances formed by green plants. Stated differently, bacteria require at least some preformed food in their dietary; they are essentially transformers, not accumulators of energy. The utilization of food by bacteria is distributed between two distinct phases of their life history, which overlap somewhat in point of time. The first is the constructive or anabolic phase, in which the parent cell, after enlarging somewhat, divides into two daughter cells, each of approximately equal size. The anabolic phase, chemically considered, is one in which the hereditary chemical complex of the protoplasm of the bacterial cell, together with the requisite enzymes and other armamentaria, is elaborated from simpler organic substances obtained from the nutritive environment.

The second, or catabolic phase, is the energy phase, in which the morphologically mature cell performs its peculiar and specific functions as a distinct biological entity. The requisite energy for the performance of these specific activities is derived from the intracellular degradation of suitable organic substances absorbed from the nutritive environment by the mature microbe. The totality of this interchange by bacteria with their nutritive environment, through which they obtain the requisite substances for their structural and energy requirements, is very properly designated bacterial metabolism. The chemistry of bacterial metabolism is the adumbration of bacterial activity.

b. The anabolic phase of bacterial metabolism. Chemical analysis of the bacterial cell reveals the presence of nitrogen, carbon, hydrogen, oxygen, together with smaller amounts of phosphorus and inorganic salts, in about the same proportions as those found in other typical cells of the animal and plant kingdoms. Phosphorus, however, is disproportionately abundant; this element, as phosphoric acid, forms the inorganic basis for nucleo protein. Nucleo protein is chemically prominent in the bacterial substance, even though not demonstrable by ordinary nuclear strains. It follows that bacterial growth depends upon the availability of these elements, both in amount and in appropriate combination.

The most significant of the essential vital structural chemical elements is nitrogen. It is strikingly inert, yet in combination with hydrogen as amino nitrogen it is the very cornerstone of life. The amino acids, of which some nineteen are known, united in a multitude of combinations, form the specific protein of all living things. With the possible exception of the nitrogen-fixing microbes and the nitrifying organisms, bacteria require, or utilize more readily, amino (or ammonia) nitrogen for their structural needs. The complexity of nitrogen combination required varies from the simplest peptids to the most complex of proteins. A few types can utilize very simple nitrogenous compounds. The great majority thrive in media containing polypeptids, or peptones. A small group of the most highly parasitized microbes, as for example the gonococcus, require, or thrive best, in media containing protein but little changed from the state in which it exists in the human or the animal body. No known microbes will develop in media from which nitrogen is excluded.

Chemically considered, the anabolic or structural phase of bacterial metabolism is a series of hydrogenic condensations in which amino acids, or complexes thereof, together with carbon, hydrogen, oxygen and inorganic salts, are woven by the microbe into the complex specific nitrogenous substance or protoplasm which determines the distinctive chemical architecture of the microbic species. Simpler carbohydrates are similarly united to form starchlike compounds, and fatty acids and glycerol, or other alcohols are united to form waxes and fats.

The actual amount of nitrogenous compounds, carbohydrates and fats entering into even very large numbers of bacteria, is small indeed. Fully fifteen millions of million typhoid bacilli would scarcely balance an ounce weight. About 85 per cent of this microbic substance is water. Even with a liberal allowance for wastes, a very small quantity

of nitrogenous substance would suffice to furnish the nitrogen of this mass of typhoid bacilli.

The anabolic or structural phase of microbic life, therefore, insofar as it can be differentiated from the energy phase, is relatively inconspicuous both with reference to the amount of chemical interchange with the nutritive environment, and with regard to the nature of the products resulting therefrom.[2]

c. The catabolic or energy phase of bacterial metabolism. The part played by bacteria in nature, their specificity of action in other words, depends very largely upon the character of the carbonaceous substances from which they obtain their energy. The amount of substance required to furnish this energy far exceeds that necessary to provide the essential structural material. The physical basis for this marked disproportion between structural and energy requirements resides largely, but not wholly, in the large surface area of the individual microbe in proportion to its volume or weight.

Two great classes of energy-containing substances are generally available for microbic catabolism, namely, the carbohydrates, and the proteins or their de ivatives. Oxidizable carbon is contained in each, but the substances resulting from the respective intracellular vital combustions of this carbon are, or may be, widely different. Thus the same organism, utilizing the carbon of a carbohydrate for its energy on the one hand, or the carbon of a peptone on the other hand, may be a veritable Dr. Jekyll or a Mr. Hyde in so far as the results of its activity are concerned. A few examples selected from a multitude of well authenticated instances of this important phenomenon will be illuminating.

The diphtheria bacillus, growing in a broth medium containing peptone and meat extractives, but no utilizable carbohydrate, produces that potent specific soluble toxin known as the diphtheria toxin. The colon bacillus cultivated in the same medium forms indol from the amino acid tryptophan. Bacillus proteus elaborates a soluble proteolytic enzyme under similar conditions. The Shiga dysentery bacillus generates a soluble poison from the same ingredients, which is said to be unlike that formed by any other microbe. Each organism, so it appears, acts characteristically and differently upon the common

[2] The poisons formed by the group of gas gangrene bacilli may prove to be exceptions. These appear during the first hours of growth and reach a maximum when the culture is multiplying most rapidly. As the culture becomes older, the poisons rapidly disappear (3).

protein constituents of the broth medium, in that the soluble products resulting from its growth are distinctive.

The same holds in the human body, where the protein of the human tissue furnishes the requisite nutriment. The diphtheria bacillus through its exotoxin incites the clinical entity, diphtheria. The Shiga bacillus is the etiologic agent of one type of bacillary dysentery. In like manner Bacillus coli forms indol in the alimentary canal from the tryptophan of the ingested protein of the food.

A striking change in the character of the products formed by these microbes takes place if some ordinary glucose is added to the respective cultures before inoculation. It must be remembered that the nitrogenous (protein) constituents of the culture medium remain quantitatively and qualitatively the same; the only change is the mere addition of a small amount of glucose. The diphtheria bacillus does not form the potent, soluble toxin in the glucose-protein medium; it produces lactic acid. The Shiga bacillus fails to make its characteristic poison; it also forms lactic acid. Indol is no longer detectable in the glucose broth culture of the colon bacillus, and no soluble proteolytic enzyme can be detected in the filtrate of the glucose broth culture of Bacillus proteus. In place of indol and the enzyme, both the colon bacillus and Bacillus proteus cultures contain acidic products, mostly lactic acid.

This remarkable transmutation of the products formed by the four microbes upon the addition of glucose to their nutritive supply is not apparently accompanied by a transformation of their structural phase; indeed, the microbes are qualitatively indistinguishable when they are tested for their specificity with their specific agglutinins, lysins or precipitins.[3] The differences, in fact, are directly attributable to the utilization of carbohydrate in place of protein for their energy requirements.

A mass of information has accumulated which indicates very clearly that utilizable carbohydrate added to cultural media protects or spares the protein of the medium from breakdown for energy among the vast majority of microbes, precisely as carbohydrate protects protein in the animal and the human body.

The effects of adding utilizable carbohydrate to cultural media as exemplified in the four bacteria just mentioned are summarized in the following table:

[3] Simonds (4) has shown, however, that there are quantitative differences in the respective agglutination, precipitation and complement fixation reactions of the plain and glucose cultures.

ORGANISM:	SIGNIFICANT PRODUCT IN PROTEIN MEDIUM:	SIGNIFICANT CHANGE IN THE CARBOHYDRATE-PROTEIN MEDIUM:
Diptheria bacillus	Soluble diphtheria toxin	Lactic acid. No toxin
Shiga bacillus	Soluble Shiga toxin	Lactic acid. No toxin
Bacillus coli	Indol	Lactic acid. No indol
Bacillus proteus	Soluble proteolytic enzyme	Lactic acid. No enzyme

It follows that the specificity of chemical action of these bacteria depends upon their utilization of protein for energy.

Conversely, when these bacteria are grown in media containing utilizable carbohydrate, they form lactic acid which is the chemical basis of buttermilk. These two generalizations are applicable to all the bacteria parasitic or pathogenic for man thus far studied with the exception of the very few that do not utilize carbohydrate for energy.

PART III. THE QUANTITATIVE MEASURE OF BACTERIAL METABOLISM. The qualitative effects of adding utilizable carbohydrate to cultures of bacteria, illustrated in the preceding section by a very few typical examples, are suggestive of the sparing action of carbohydrates for protein, but they fail to afford satisfying quantitative substantiation of the general principle involved. Physiologists have long been conversant with this same principle as it is manifested in animal and human metabolism. Howell (5) expresses it thus: "The oxidization of the sugar protects the protein of the body." There seems to be little doubt that the sparing action of carbohydrate for protein is a rather general principle of the metabolism of cells in general which require preformed food in their dietary. If such be the case, the study of bacterial metabolism should reveal the pattern upon which the fundamental metabolism of animal and animal-like cells is moulded.

Suitable quantitative chemical methods for the exploration of this field have been perfected by Folin and his associates.[4] It is not without significance in this connection that these procedures were developed by Folin for the study of human metabolism.

The quantitative study of the nitrogenous changes induced in culture media of predetermined composition by many kinds and strains of bacteria has revealed the basis of the phenomena of bacterial metabolism (7) and has indicated the parallelism between microbic (unicellular) and animal and human (polycellular) metabolism. Space does not permit of an extensive discussion of the metabolism of the various

[4] See Kendall (6) for those especially adapted to the study of bacterial metabolism.

types of bacteria, but for convenience of discussion the protocols of the nitrogenous metabolism of two representative organisms are reproduced, namely, B. typhosus (8) and B. proteus (9):

TABLE 1

Bacillus typhosus, 1

MGM. PER 100 CC.	CONTROL	DAY	PLAIN BROTH	GLUCOSE BROTH
Total nitrogen	1.080	1	1.080	1.080
Protein nitrogen	0.778		0.800	0.790
Nonprotein nitrogen	0.302		0.280	0.290
Polypeptid nitrogen	0.210		0.175	0.196
Amino nitrogen	0.042		0.048	0.045
Ammonia nitrogen	0.050		0.057	0.049
Reaction	+0.80		+1.30	+5.10
pH	7.2		7.3	5.0
Total nitrogen	1.080	4	1.080	1.080
Protein nitrogen	0.778		0.811	0.801
Nonprotein nitrogen	0.302		0.269	0.279
Polypeptid nitrogen	0.210		0.167	0.181
Amino nitrogen	0.042		0.044	0.051
Ammonia nitrogen	0.050		0.058	0.047
Reaction	+0.80		−0.80	+5.40
pH	7.2		7.8	5.0
Total nitrogen	1.080	7	1.080	1.080
Protein nitrogen	0.778		0.923	0.913
Nonprotein nitrogen	0.302		0.157	0.167
Polypeptid nitrogen	0.210		0.044	0.067
Amino nitrogen	0.042		0.043	0.052
Ammonia nitrogen	0.050		0.070	0.048
Reaction	+0.80		−1.40	+5.80
pH	7.2		8.4	5.0
Total nitrogen	1.080	10	1.080	1.080
Protein nitrogen	0.778		0.868	0.890
Nonprotein nitrogen	0.302		0.212	0.190
Polypeptid nitrogen	0.210		0.097	0.102
Amino nitrogen	0.042		0.038	0.040
Ammonia nitrogen	0.050		0.077	0.048
Reaction	+0.80		−2.10	+4.20
pH	7.2		8.7	5.3

DISCUSSION OF ANALYTICAL TABLES. The methods used in these studies, developed for the most part by Folin and his associates (6),

are of sufficient precision to warrant the supposition that the quantitative changes induced by the bacteria in the nitrogenous constituents of the cultural media furnish a fairly accurate outline of the nitrogenous changes taking place therein during the cultural history of the respective microbes selected for discussion.[5]

Bacillus typhosus:[6] The general phenomena indicative of the sparing action of the glucose for the protein are quite clearly discernible. The reaction of the plain, sugar-free medium (plain broth) becomes progressively alkaline, and, also, the hydrogen ion concentration diminishes progressively. Coincidently, the ammonia detectable in the culture increases from 50 mgm. per 100 cc. medium in the control, to 77 mgm. at the end of ten days' incubation. The increasing alkalinity is presumably attributable to the gradual formation of basic substances. In glucose broth, on the other hand, which has precisely the same nitrogenous composition and reaction, but to which 1 per cent of glucose has been added, the reaction becomes increasingly acid. Both the titratable acid and the hydrogen ion concentration show a rapid and decided increase. This follows from the well attested fact that the products formed from the utilization of sugar for energy are acidic in character and reaction. The free ammonia, however, undergoes practically no change in the glucose broth. Inasmuch as ammonia results for the most part from the deamination of the protein derivatives within the bacterial cell prior to the utilization of the non-nitrogenous residuum of the protein derivative for energy, this disproportion between free ammonia in plain broth and glucose cultures of the organism might be expected.

It is of some interest to determine what type of nitrogenous substance of the culture medium undergoes the most noteworthy change during the growth of the bacteria. A survey of the table shows clearly that the "polypeptid" nitrogen fraction (see reference (6) for detailed explanation) is chiefly altered. The polypeptid nitrogen decreases in amount somewhat more rapidly in the plain broth than the glucose broth during the first week of growth. Then it increases again. This increase is probably due to autolysis of some of the bacteria at the later period, when cultural conditions have become rather unfavorable

[5] For additional studies, see Journ. Infect. Dis., 1922, xxx, no. 2. These appear to be the first complete quantitative investigations of the nitrogenous metabolism of cellular activity recorded.

[6] The analytical figures for the various nitrogen fractions in this and the following tables are expressed in fractions of a gram per 100 cc. culture medium.

for growth. It will be noted that there is a simultaneous increase in the "protein nitrogen" fraction during the first seven days of incubation. This is clearly attributable to the incorporation of some of the "polypeptid nitrogen" into the bodies of the newly formed bacteria. After the seventh day the protein fraction diminishes somewhat, evidence again of autolysis of some of the bacteria. The amino nitrogen does not undergo changes of sufficient magnitude to become of significance.

Bacillus proteus: Tables 2 and 3 are reproduced, (pp. 448–449) the first showing the changes induced in plain broth and in the same plain broth to which graded amounts of glucose have been added; the second table shows the changes induced by sterile filtrates of the various broths shown in table 2 in a sterile broth medium.[7] It will be remembered that Bacillus proteus forms soluble proteolytic enzymes in media from which considerable amounts of utilizable carbohydrates are excluded. The effect of the increasing amounts of glucose up to 1.5 per cent, at which point the enzyme does not appear in an active state, is clearly indicated. The sparing action of the glucose up to a concentration of 1.5 per cent, which is more than the bacteria can completely oxidize, even in fourteen days, is indicated by the slowness of the action of the enzyme in the higher sugar concentrations during the earlier days of incubation.

The sparing action of the glucose for the protein is also indicated by the very considerable amount of ammonia formed both in the plain broth culture and those containing relatively small amounts of glucose, in contrast to the negligible amount of free ammonia in the 1.5 per cent glucose culture. In the former, the ammonia increase amounts to as much as 200 mgm. per 100 cc. of culture medium after two weeks' incubation. In the latter (1.5 glucose) it amounts to but 6 mgm., a truly striking difference.

Comparing the plain broth culture with the 1.5 glucose broth culture the changes in the "polypeptid nitrogen" are noteworthy. In the former, the polypeptid nitrogen *increases* from 280 mgm. to as much as 963 mgm., whereas in the glucose culture a small *decrease* is clearly discernible. The difference is due to the presence of the soluble enzyme in the plain sugar-free broth culture. This is indicated by the *decrease* in the protein nitrogen fraction from 721 mgm. to 38 mgm. in the plain broth culture, notwithstanding the fact that there has been a considerable amount of protein nitrogen bound up in the bodies of the bacteria. At the end of the seventh day apparently practically

[7] Full details of the experiment will be found in reference (9).

TABLE 2

B. proteus

PROTEUS NO. 1 JUNE 28, 1921 CULTURES	DAY	CONTROL	PLAIN	PERCENTAGE OF GLUCOSE						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen	1	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.531	0.564	0.598	0.609	0.665	0.676	0.721	
Nonprotein nitrogen		0.280	0.470	0.437	0.403	0.392	0.336	0.325	0.280	
Polypeptid nitrogen		0.209	0.389	0.361	0.330	0.319	0.265	0.253	0.213	
Amino nitrogen		0.039	0.032	0.028	0.028	0.029	0.030	0.032	0.029	
Ammonia nitrogen		0.032	0.049	0.048	0.045	0.044	0.041	0.040	0.038	
Total nitrogen	2	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.262	0.195	0.385	0.419	0.453	0.497	0.497	0.743
Nonprotein nitrogen		0.280	0.739	0.806	0.616	0.582	0.548	0.504	0.504	0.258
Polypeptid nitrogen		0.209	0.638	0.709	0.527	0.415	0.475	0.429	0.434	0.191
Amino nitrogen		0.039	0.032	0.030	0.029	0.029	0.028	0.027	0.026	0.030
Ammonia nitrogen		0.032	0.069	0.067	0.060	0.048	0.045	0.048	0.044	0.037
Total nitrogen	3	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.239	0.239	0.284	0.352	0.430	0.453	0.531	
Nonprotein nitrogen		0.280	0.762	0.762	0.717	0.649	0.571	0.548	0.470	
Polypeptid nitrogen		0.209	0.647	0.647	0.608	0.536	0.473	0.469	0.400	
Amino nitrogen		0.039	0.036	0.036	0.032	0.030	0.047	0.030	0.028	
Ammonia nitrogen		0.032	0.089	0.089	0.077	0.056	0.051	0.049	0.042	
Total nitrogen	4	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.060	0.073	0.139	0.228	0.262	0.307	0.351	
Nonprotein nitrogen		0.280	0.941	0.928	0.862	0.773	0.739	0.694	0.650	
Polypeptid nitrogen		0.209	0.796	0.777	0.709	0.696	0.654	0.613	0.571	
Amino nitrogen		0.039	0.028	0.035	0.035	0.018	0.035	0.032	0.030	
Ammonia nitrogen		0.032	0.117	0.116	0.118	0.059	0.050	0.049	0.049	
Total nitrogen	5	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.161	0.127	0.161	0.206	0.206	0.239	0.340	0.822
Nonprotein nitrogen		0.280	0.840	0.847	0.840	0.795	0.795	0.762	0.661	0.179
Polypeptid nitrogen		0.209	0.663	0.694	0.679	0.654	0.667	0.648	0.567	0.103
Amino nitrogen		0.039	0.033	0.031	0.032	0.024	0.025	0.035	0.030	0.039
Ammonia nitrogen		0.032	0.144	0.149	0.129	0.117	0.103	0.079	0.064	0.037
Total nitrogen	7	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001
Protein nitrogen		0.721	0.038	0.094	0.071	0.060	0.083	0.094	0.105	0.732
Nonprotein nitrogen		0.280	0.963	0.907	0.930	0.941	0.918	0.907	0.896	0.269
Polypeptid nitrogen		0.209	0.696	0.687	0.703	0.757	0.750	0.730	0.722	0.185
Amino nitrogen		0.039	0.036	0.025	0.032	0.035	0.042	0.040	0.033	0.044
Ammonia nitrogen		0.033	0.204	0.195	0.195	0.149	0.126	0.137	0.141	0.039
Total nitrogen	14	1.001	1.001	1.001	1.001	1.001	1.001	1.001	1.001	
Protein nitrogen		0.721	0.162	0.151	0.140	0.218	0.151	0.106	0.106	
Nonprotein nitrogen		0.280	0.839	0.850	0.861	0.783	0.850	0.895	0.895	
Polypeptid nitrogen		0.209	0.587	0.577	0.566	0.511	0.580	0.600	0.624	
Amino nitrogen		0.039	0.039	0.026	0.030	0.059	0.057	0.079	0.050	
Ammonia nitrogen		0.033	0.213	0.247	0.265	0.213	0.213	0.216	0.221	

TABLE 3

B. proteus

CARBOL GELATIN ENZYME	DAY	CONTROL	PLAIN	PERCENTAGE OF GLUCOSE						
				0.1	0.2	0.3	0.4	0.5	0.75	1.5
Total nitrogen	1	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen		0.542	0.325	0.280	0.381	0.392	0.426	0.426	0.471	
Nonprotein nitrogen		0.130	0.347	0.392	0.291	0.280	0.246	0.246	0.201	
Polypeptid nitrogen		0.102	0.314	0.360	0.257	0.252	0.217	0.217	0.172	
Amino nitrogen		0.020	0.025	0.024	0.028	0.022	0.023	0.023	0.023	
Ammonia nitrogen		0.008	0.008	0.008	0.006	0.006	0.006	0.006	0.006	
Total nitrogen	2	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672
Protein nitrogen		0.542	0.235	0.246	0.314	0.403	0.414	0.358	0.370	0.515
Nonprotein nitrogen		0.130	0.437	0.426	0.358	0.269	0.258	0.314	0.302	0.157
Polypeptid nitrogen		0.098	0.384	0.379	0.314	0.235	0.227	0.285	0.273	0.125
Amino nitrogen		0.023	0.044	0.038	0.035	0.026	0.022	0.022	0.020	0.023
Ammonia nitrogen		0.009	0.009	0.009	0.008	0.008	0.009	0.007	0.009	0.009
Total nitrogen	3	0.672	0.672	0.672	0.672	0.672	0.672	0.672	0.672	
Protein nitrogen		0.542	0.078	0.112	0.146	0.202	0.258	0.291	0.347	
Nonprotein nitrogen		0.130	0.594	0.560	0.526	0.470	0.414	0.381	0.325	
Polypeptid nitrogen		0.097	0.534	0.498	0.475	0.430	0.368	0.344	0.294	
Amino nitrogen		0.023	0.048	0.052	0.042	0.031	0.038	0.029	0.023	
Ammonia nitrogen		0.010	0.012	0.010	0.009	0.009	0.008	0.008	0.008	
Total nitrogen	4	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	
Protein nitrogen		1.061	0.389	0.447	0.524	0.515	0.793	0.793	0.804	
Nonprotein nitrogen		0.269	0.941	0.883	0.806	0.815	0.537	0.537	0.526	
Polypeptid nitrogen		0.119	0.860	0.819	0.737	0.459	0.484	0.484	0.478	
Amino nitrogen		0.035	0.065	0.048	0.053	0.041	0.038	0.037	0.032	
Ammonia nitrogen		0.015	0.016	0.016	0.016	0.015	0.015	0.016	0.016	
Total nitrogen	5	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen		1.061	0.322	0.332	0.330	0.390	0.424	0.547	0.670	1.051
Nonprotein nitrogen		0.269	1.008	1.008	0.940	0.940	0.906	0.783	0.660	0.279
Polypeptid nitrogen		0.211	0.916	0.907	0.844	0.847	0.812	0.700	0.575	0.222
Amino nitrogen		0.040	0.073	0.083	0.078	0.075	0.077	0.066	0.067	0.040
Ammonia nitrogen		0.018	0.019	0.018	0.018	0.018	0.017	0.017	0.018	0.017
Total nitrogen	7	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330
Protein nitrogen		1.061	0.367	0.390	0.333	0.547	0.524	0.524	0.524	0.983
Nonprotein nitrogen		0.269	0.963	0.940	0.997	0.883	0.806	0.806	0.806	0.347
Polypeptid nitrogen		0.205	0.861	0.832	0.884	0.778	0.706	0.706	0.708	0.279
Amino nitrogen		0.039	0.076	0.082	0.086	0.081	0.076	0.075	0.073	0.043
Ammonia nitrogen		0.025	0.026	0.026	0.027	0.024	0.024	0.025	0.025	0.025
Total nitrogen	14	1.330	1.330	1.330	1.330	1.330	1.330	1.330	1.330	
Protein nitrogen		1.061	0.413	0.547	0.402	0.402	0.367	0.379	0.469	
Nonprotein nitrogen		0.269	0.917	0.883	0.928	0.928	0.963	0.951	0.861	
Polypeptid nitrogen		0.191	0.821	0.738	0.820	0.826	0.854	0.847	0.750	
Amino nitrogen		0.049	0.067	0.070	0.078	0.073	0.082	0.076	0.082	
Ammonia nitrogen		0.029	0.029	0.030	0.030	0.029	0.027	0.028	0.029	

all of the original protein nitrogen has been broken down to the non-protein nitrogen fraction by the enzyme, leaving only that protein locked up in the newly formed bacteria. Strong supportive evidence of this view is found not only in the autolytic changes taking place in the fourteen-day cultures, but also in the 1.5 glucose broth culture, where no enzyme is present. Here the protein fraction *increases* up to the seventh day, while the non-protein fraction *decreases*, precisely as was found to be the case with Bacillus typhosus, which forms no soluble enzyme.

Turning to the changes induced by the soluble, bacteria-free enzyme, it will be found that a transfer from the protein to the non-protein nitrogen takes place to a very considerable extent—amounting to a change from 1061 mgm. to 322 mgm. in the fifth day. It should be remembered that only 5 per cent of the normal amount of enzyme found in the cultures of this date has been added to the sterile broth medium; this indicates the activity of the enzyme.

In glucose broth where no enzyme is present, there is no change of any significance in the nitrogenous constituents; this should be expected. One feature deserves special mention. In no instance where the active enzyme (bacteria-free) is added to the sterile medium is there the slightest increase in free ammonia, notwithstanding the great decrease in protein nitrogen. The free ammonia, as indicated above, is associated with the intra-bacterial utilization of the protein for energy. The soluble enzyme is wholly without deaminizing properties.

Not very much "amino nitrogen" is formed by the action of the soluble enzyme upon the protein constituents of the medium. This would suggest that the action of the enzyme is more like that of pepsin than that of trypsin or erepsin. In other words, the soluble enzyme of Bacillus proteus does not reduce the protein nitrogen fraction or the polypeptid fraction of the culture medium to the state of single, free amino acids. Rather, there is a cleavage of the proteins and the more complex protein derivatives to polypeptids of unknown complexity.

These analytical studies of the nitrogen distribution in microbic cultures furnish but little information of the identity of the products formed during the life history of the microbe, but they indicate quite definitely the quantitative character of the nitrogenous interchange between medium and microbe as growth proceeds. The metabolism of the human body is studied in precisely the same manner, and with similar methods.

It is not without significance that the diphtheria bacillus, growing either in the human body or in the glass test tube, produces the same toxin. The nitrogenous tissues of the body and the nitrogenous constituents of the culture medium alike yield the same by-product—diphtheria toxin—when they are used for energy by the diphtheria bacillus. On the other hand, the bacteria that produce their harmful effects only when they develop within the tissues of the body, as typhoid, streptococci and others, present a problem that is still obscure. The elucidation of the nature of the products of growth formed by pathogenic bacteria is one of the rich prizes this field holds forth for the future.

Notwithstanding the indefiniteness of current information regarding the chemical composition of the products formed during nitrogenous metabolism by bacteria within the body and in culture media, the general principle of the sparing action of utilizable carbohydrate for protein has been revealed by these quantitative metabolic studies, and bacterial metabolism has been brought into accord with the general phenomena of metabolism in the animal body.

PART IV. CERTAIN APPLICATIONS OF BACTERIAL METABOLISM. In a preceding section attention was directed to the dissimilarity of products formed from the common ingredients of ordinary, sugar-free cultural media by various parasitic and pathogenic bacteria. In other words, each microbe exhibits a marked specificity of chemical action, judging from the nature of the substances formed when each utilizes protein or protein derivatives for energy. Conversely, each of these respective microbes forms acidic products, chiefly or commonly lactic acid, when it utilizes carbohydrate for its energy requirements.

Not all carbohydrate configurations are utilizable for bacterial energy. Indeed, a very striking specificity exists between the protoplasmic asymmetry of bacteria and their respective abilities to utilize particular sugar configurations. The tissues of the human body are equally exacting in this respect.

This important relationship between protoplasmic asymmetry and the stereo-configuration of utilizable carbohydrate and carbohydrate-like substances has long been a theme for investigation. Pasteur (10) showed many years ago that a mold, Penecillium, would ferment the dextro component of ammonium racemate, leaving the laevo isomer practically intact, and this method of separating isomers by the action of biological agents was rapidly extended (11). Fischer and Thierfelder (12) examined the relations between the stereo-configurations

of certain sugars and their fermentability by yeast. From this study, and subsequent investigations upon a more extensive series of sugars and glucosides, Fischer drew his famous simile that a carbohydrate, to be acted upon, must possess a stereo-configuration that is related to the asymmetry of the cellular protoplasm as a "key is to its particular lock" (13). Subsequent studies have thrown additional light upon the interrelations of bacteria and various carbohydrates. Out of these interrelations procedures have been devised which permit of of the microbic identification and estimation of various sugars and sugar derivatives (14). The correctness of the underlying principle, that "utilizable carbohydrate spares protein from utilization for energy" (15), seems to be adequately established by the very operation of this use of bacteria in carbohydrate analysis.

Many problems of interest and importance present themselves as the direct outcome of the mutual interdependence of microbic nutrition and potential microbic action. Not the least interesting and important of these is the multitude of phenomena having their origin in the alimentary canal of man. Volumes have been written upon this subject. At most, a most superficial outline can be presented here.

From birth to normal adult life the kaleidoscopic changes in the flora of the intestinal tract have their origin in the general alimentary environment (16). The food of the host is a very important factor, both in controlling the kinds of bacteria that multiply in the intestinal canal, and in determining the nature of the products these bacteria form while they are resident therein. Thus, the monotonous breast milk diet of the nursling with its preponderance of lactose creates conditions that favor the growth of the anaerobic, obligately fermentative organism, Bacillus bifidus (17). This microbe is devoid of more than minimal proteolytic powers; indeed, it thrives in the alimentary tract only when available lactose is continuously present. As the child grows and its dietary requirements exceed the maternal ability to provide, a noteworthy change is discernible in the intestinal flora, coincident with the altered nutritional conditions. The dietary change is essentially a marked increase in the proportion of protein to carbohydrate, which usually results in a differential enrichment of the protein residuum in the lower portion of the child's intestinal tract, with periods of carbohydrate presence and absence. Under these conditions, Bacillus bifidus tends to disappear, being unable to thrive in the periodic absence of carbohydrate, and more versatile bacteria take its place. Bacillus coli is the most conspicuous of these. It can grow well upon

the partly digested protein residuua, and utilize the protein for energy during those periods when sugar is absent. That is to say, Bacillus coli, unlike Bacillus bifidus, is facultative with reference to its energy requirements (18). It is worthy of note that considerable amounts of indol are formed by it when it utilizes protein for energy, whereas it produces lactic, and smaller amounts of other acidic products when utilizable carbohydrate is available. The indol, absorbed from the alimentary tract, oxidized and paired in the liver, is a principal source of urinary indican.

This change from an obligately or nearly obligately fermentative nursling flora to a facultative putrefactive flora persists from post nursling life through adolescence to adult life. Inasmuch as experimental animals may be made to exhibit the major features of this bacterial change in type and chemical activity through careful dietary alternations (16), (17), (19), it would appear not only that diet is one important factor in determining the nature of the intestinal flora, but also that suitable dietary procedures offer a rather direct method of influencing the nature of the products formed by bacteria already present in the alimentary tract.

It is quite obvious that undesirable intestinal microbic activity may be of at least two types—one in which harmful protein derivatives are being formed, as for example in bacillary dysentery or in an overgrowth of bacteria which produce intolerable amounts of indol, or other substances that may act as slowly cumulative systemic poisons. The other type is characterized either by irritant acidic substances, as butyric acid in considerable amounts, or to unusual amounts of lactic or acetic acids.

Two fairly distinct procedures have been advocated to influence unfavorable bacterial activity in the alimentary canal. One of these consists essentially in forcing dietary procedures inimical to the growth of the microbe it is desired to supplant; the other contemplates the deliberate introduction of bacteria whose activities are of necessity favorable to the well-being of the host.

Feeding diets rich in lactose have been found helpful in infections with dysentery bacilli (20) and in typhoid fever (21). Here the primary object is to furnish the invading bacteria, and intestinal bacteria as well, with a continuous supply of carbohydrate, in order to shift the metabolism from the protein to the carbohydrate, and thus change the bacterial products formed in so far as possible to the lactic producing phase. The results of this method of therapy are quite encouraging.

In a similar manner, Dragstedt (22) has been able to produce and control parathyroid tetany in experimental animals in a very suggestive manner; upon a protein diet, the animals manifest the typical signs and symptoms of this disease; the substitution of a diet rich in carbohydrate ameliorates the symptoms very markedly.

The second method—that of deliberate intestinal implantation of suitable bacteria—is as yet not well worked out but available evidence indicates that Bacillus bifidus, or Bacillus acidophilus (23), freshly isolated from the intestinal tract, so that the ability to grow in the intestinal environment is not lost, may be fed in the living state, and with suitable accessory dietary conditions may be induced to grow in the intestines. It is clear from what has been said that the implantation and growth of bifidus or acidophilus in the alimentary canal takes place only under conditions that will afford optimum conditions for the normal resident bacteria to change their metabolism to the lactic acid phase. Hence, growth and persistence of the artificially implanted bacteria is rather an index of the persistence of carbohydrate throughout the alimentary tract, with its effect upon the nature of their products of metabolism, than merely a successful inoculation with the foreign microbe. In no case is it possible to replace all the normal intestinal bacteria with bifidus and acidophilus cultures, except in very young children and animals.

The study of the chemistry of bacterial metabolism—and this is equally true for cellular metabolism in general—is in its formative state. Advances along highly important lines of the identification of individual growth products must await the development of new chemical methods. The ultimate results will carry biological science far along the path toward a real understanding of the dynamics of life itself.

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